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DIETARY FIBER AND PROTEIN FROM PLANT AND ANIMAL ORIGIN

Intake patterns in Europe and China and their association with body composition, socio-economic status and lifestyle during the period of 2002-2007

Yi Lin

Thesis submitted in fulfilment of the requirements for the degree of Doctor in Medicine and Health Sciences

Prof. Dr. Stefaan De Henauw (Promoter)

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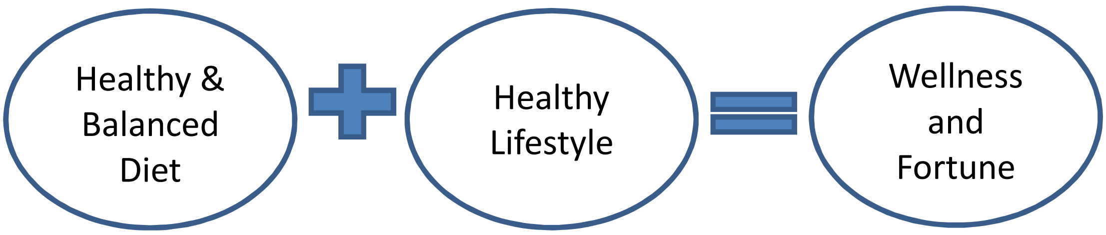
A healthy and balanced diet is the best medicine.



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Abbreviations

AOAC	Association of Analytical Chemists
BF%	body fat percentage
BLS	the German Food Code and Nutrient Data Base
BMI	body mass index
BNFCS	the Belgian national food consumption survey
BP	blood pressure
BSHC	the Belgian Superior Health Council
CHD	coronary heart disease
CHNS	the China health and nutrition survey
CI	confidence interval
CRP	C-reactive protein
CVD	cardiovascular diseases
DAFNE	the Data Food Networking
DASH	Dietary Approaches to Stop Hypertension
DBP	diastolic blood pressure
DF	dietary fiber
DIAT	dietary assessments tool
E%	energy percentage
EDR	estimated dietary records
EFCOSUM	the European Food Consumption Survey Method project
EFSA	the European food safety authority
ELISA	Enzyme-Linked ImmunoSorbent Assay
EPIC-SOFT	European Prospective Investigation into Cancer and Nutrition software
FBDG	the Food Based Dietary Guidelines
FDA	US Food and Drug Administration
FPDS	the Flanders preschool dietary survey
FM	flour (pasta)-rich meals
GLM	generalized linear model
HDL-C	high-density lipoprotein cholesterol
HELENA	the Healthy Lifestyle in Europe by Nutrition in Adolescence
HELENA-CSS	the Healthy Lifestyle in Europe by Nutrition in Adolescence - Cross-Sectional Study
HP	high protein

HPHF	high protein and high fiber
HM	heavy meals (incl. energy-dense foods and oil)
HS	the region of hot and spicy foods
IARC	International Agency for Research on Cancer
IEL	Institut für Ernährungs- und Lebensmittelwissenschaften
INRAN	Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione
IOM	Institute of Medicine
IOTF	the International Obesity Task Force
LCHP	a low-carbohydrate/high-protein diet
LDL-C	low-density lipoprotein cholesterol
LFHC	a low-fat, high-carbohydrate diet
LFLC	low-fat/low-calorie diets
MetS	metabolic syndrome
LPHC	low-protein/ high-carbohydrate diet
MANCOVA	multiple analyses of covariance
MLM	mixed linear models
MSM	Multiple Source Method
NHANES	the US National Health and Nutrition Examination Survey
NP	normal protein
NSP	non-starch polysaccharides
NS	not statistically significant
OW	overweight
OB	obesity
SBP	systolic blood pressure
SD	standard deviation
SE	standard error
SEM	standard error of mean
SES	socio-economic status
SP	standard-protein
SPSS	Statistical Package for the Social Sciences
SM	sweet but less heavy meals
T2D	type 2 diabetes
TC	total cholesterol
TG	triglyceride
RCT	randomized controlled clinical trials

RDA	recommended dietary allowances
USDA	US Food and Drug Administration
VLDL-C	very low-density lipoprotein cholesterol
VM	variety meals (often with sour taste)
WC	waist circumference
W/H	waist-to-height ratio
WHR	waist-to-hip ratio
WIF	water-insoluble fiber
WSF	water-soluble fiber
WHO	World Health Organization
YANA-C	the Young Adolescents' Nutrition Assessment on Computer

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PREFACE

A well-balanced, healthy diet with a variety of food items supplies all the nutrients required for an optimal nutritional status. Yet, food behaviors are strongly associated with culture and personal lifestyles. In Western countries, energy-dense foods like red meat, sweets and desserts are consumed in higher quantities than fruits and vegetables which result in high energy, saturated fat, sugar and salt intakes. Those high energy intakes, thus, eventually lead to obesity (OB) and its concomitant disorders like cardiovascular diseases (CVDs), metabolic syndrome (MetS), type 2 diabetes (T2D), stroke, kidney diseases and other chronic, non-communicable diseases (NCD). However, in East Asian countries like China and Japan, the incidence rates for OB, CVD and coronary heart disease (CHD) are still much lower compared to Western countries. The different dietary behaivours, such as consuming much higher plant products, can be an important factor to explain the phenomenon. However, because of Westernization, innovative food intakes and lifestyle, the Asian culture has been changing rapidly and has created new modern food habits and lifestyles, resulting in a “nutrition transition”. Increasing fat and cholesterol intakes, and decreasing intakes of traditional plant based foods, coincide with an increased sedentary lifestyle, leading to OB and OB-related NCD causing a relatively higher incidence of chronic diseases.

Dietary protein and fiber, used in clincial trials and longitudinal studies in the past decades, show protective effects against the development of chronic diseases. Both dietary protein (10-15% of total enery intake) and fiber intakes have been proven to affect positively on regulation of energy intake and increment of satiety [197;449], leading to the ultimate reduction in body weight [140;164;183;476;495] and improvement of blood lipid profiles [53;212;459] in the long-run. However, high dietary protein intake (≥ 15% of total every intake) may lead to negative health outcomes [193;263]. The significantly different impacts between animal and plant protein on OB and chronic diseases are the concomittant intakes of fat and cholesterol. In addition, the two types of dietary fibers,water-soluble fiber (WSF) and water-insoluble fiber (WIF), have different mechanisms in their impact on the human health. WSF can delay small-bowel absorption, which may reduce cholesterol absorption, but also pancreatic enzyme activity and protein digestion. Subsequently, the colonic fermentation of fibers results in the production of gases and short-chain fatty acids, which undoubtedly contributes to a lower glycemic index of foods, and, consequently, attenuates the insulin response [156;474]. Due to its anti-nutritive properties and non-digestibility, WIF can increase the bulkiness of stool and faecal mass, thereby shortening transit time.

Generally, plant-based foods may protect more against OB and its concomitant chronic diseases including heart diseases, high cholesterol, high blood pressure (BP), and T2D, compared to a diet that contains more energy, meat and fat.

CHAPTER 1

GENERAL INTRODUCTION

1. BACKGROUND

The past decades have seen a dramatic increase in OB prevalence worldwide, leading to a global epidemical concern [241;547]. This has resulted in a concomitant increase in chronic diseases, such as MetS, hypertension and stroke, CVD, T2D, and certain cancers. The definition of OB is a condition of 'abnormal or excessive fat accumulation in adipose tissue to the extent that health may be impaired' [541]. The World Health Organization (WHO) defined the cut-off values for OW and OB in Western adults as 25.0–29.9 kg/m^2 and ≥30.0 kg/m^2, respectively [542], which is different for Asian populations and migrant Asian populations in Western countries (OW: 23.0–27.5 kg/m^2; OB: ≥27.5 kg/m^2) [221;353;553]. The definitions of OW and OB for children and adolescents are different than that for adults, and are based on BMI z-score cut-off values as proposed by Cole *et al.* [102;103].

Globally, over 42 million children under the age of 5 were estimated to be OW and OB in 2013 [550]. WHO reported more than 1.9 billion OW adults, of which 600 million considered obese, globally by the end of 2014 [550]. At least 2.8 million of these people die every year worldwide due to OW and OB [549]. In 2011-2012, 17.7% and 20.5% of US children and adolescents, respectively, were defined OB and 69.0% of the adults were OW, including 35.1% OB [90]. The estimated numbers of OW and obese European children increased by approximately 1.3 million children per year by the end of 2010 [239] and the rates of OB were increasing with up to 36% in some European regions [241]. Over 50% of both men and women in Europe in 2008 were diagnosed as OW, and roughly 23% of women and 20% of men were obese [549]. Likewise, many Asian countries such as China and India are facing challenges of OB due to over-nutrition. In China, the prevalence of OW and OB in children and adolescents aged 6 to 19 years old in big cities such as Beijing, Shanghai has reached 17.7% and 14.4%, respectively, which has been observed to be close to western countries [556]. Between 1993 and 2009, the prevalence of general OB in Chinese adults increased with 8.5% and 5.1% in men and women, respectively [560]. While the prevalence of abdominal OB increased from 8.5 to 27.8% among men and from 27.8 to 45.9% among women during two decades [560].

The burden of chronic diseases is rapidly increasing worldwide. The increase in morbidity and mortality due to OB and its concomitant chronic diseases has become a global concern. More than 115 million people suffer from OB and OB-related health problems [547], e.g.

CVD is already the leading cause of mortality in many developing countries, like India and China. In addition, it has been projected in developing countries, by 2020, almost 75% of all deaths worldwide will be attributed to chronic diseases, and that 71% of deaths due to ischaemic heart disease, 75% of deaths due to stroke, and 70% of deaths [548].

Diet is a critical factor in promoting and maintaining optimal health in both children and adults. For decades, diet has been known to play a key role as a risk factor for the development of OB and chronic diseases [548]. Evidence shows that nutritional intakes during early years are significantly associated with OB in adolescence with a further impact on adult OW and OB [309;478]. The nutrition transition is characterized by a shift in dietary consumption and energy expenditure with economic, demographic, and epidemiological changes (Figure 1.1.). In the Western world, the nutrition transition took place some decades ago and has led to the typical Western dietary pattern rich in high-fat-energy-dense diet [396], one of the most likely fundamental causes of the OB. These high-fat-energy-dense diets, together with sedentary lifestyle, have led to a dramatic elevation in the prevalence of OB [477], which is often observed in rural areas and among those with low socio-economic status (SES). Recent nutrition transition is referring to developing countries (e.g. China, India) from relatively healthy diets of varying traditional diets, based on whole grains or roots, legumes, vegetables and fruits, and limited foods of animal origin, towards a relatively industrialized diet, including more prepressed food, food of animal origin, sugar and fat, beverages, and often more alcohol [38;573;574]. This nutrition transition resulted in an increased risk of OB and its concomitant diseases among Asian populations.

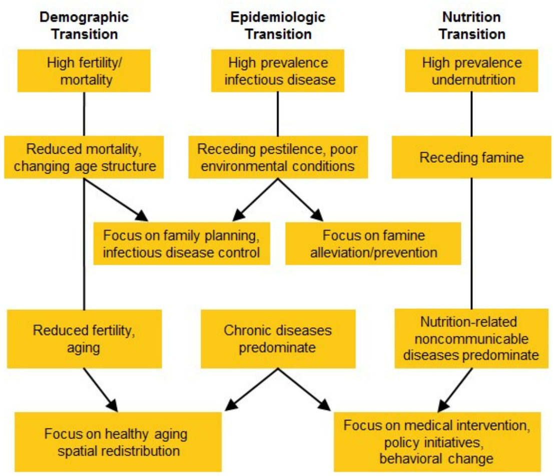

Figure 1.1 Health, Nutrition, and Demographic Change [397].

Debate continues regarding the most appropriate macronutrients such as protein and carbohydrates, and other dietary components like dietary fiber (DF) that could assist in the prevention of OB, CVD and T2D [368;385]. Research on the prevention and treatment of OW and OB suggests that relatively high protein (HP) and DF diets might be acting on different metabolic targets regulating body weight, maintaining body composition and improving lipid-lipoprotein profiles [340;476].

Recent reviews suggest that diets rich in protein and DF may be beneficial in protecting against OW, OB, and its concomitant diseases among adults and children [223;362]. Previous studies with different study designs including randomized trials and longitudinal studies showed that diets rich in plant protein have more health benefits than those based on animal proteins via weight loss, improving blood lipid profiles and adjusting fasting glucose and insulin resistance [14;272;366;369]. The changed dietary patterns in Asian countries due to nutrition and lifestyle transition towards a Westernized lifestyle [573] resulted in increases in OB prevalence and the related consequences in the whole Chinese population [249;560].

When considering the food sources, namely animal versus plant protein intakes, on OB, and its concomitant diseases, findings from observational studies and clinical trials indicated more health benefits from plant protein intake than animal protein intake to achieve weight loss, and improve body composition, lipid-lipoprotein profile and BP [72;246;496;522].

2. DIETARY PROTEIN

2.1. Definitions and dietary sources

Dietary protein, made of amino acids to form peptides, is an essential nutrients part of the diet, ideally contributing 10-15% to the total energy supply, as recommended by WHO [551]. Hence, normal protein (NP) and HP need to be defined in both relative (percentage energy) and absolute (g/protein) terms based on energy intake and energy expenditure. Relatively HP intake need to be consumed at least 20–30% of total energy [530]. Due to differences in not only amino acid composition, but also digestibility between animal and plant proteins, these two protein types act different and should, therefore, be assessed separately [274;326]. Sources of animal protein are mainly from dairy products, meat and meat products, fish and shellfish, and eggs, while plant proteins are mainly from potatoes and other tubers, vegetables, legumes, fruits, nuts and seeds, cereals and cereal products (Table 1.1.).

Table 1.1. List of protein contents (g protein/ 100g) from main food sources

Food sources $^{\mu}$	Protein (g/ 100g)
Fresh Meat (raw)	
Pork chop	19.4
Lamb chop	20.4
Chicken without skin,	20.5
Veal steak	21.8
Beef, lean	22.8
Prepared meat/cold cut	
Ham	20.7
Salami	21.1
Smoked beef	22.9
Fish, molluscs and crustaceans	
Codfish	16.6
Coalfish	17.2
Salmon	18.4
Tuna	27.4
Sardines in oil, canned	23.2
Whole egg, raw	12.6
Milk and yogurt	
Semi-skimmed milk	3.3

Low-fat yogurt	4.5
Cheese	
Low-fat cottage cheese	7.6
Cheese, Camembert 45+	21.7
Cheese, Gouda	25.3
Cheese, Emmental	28.7
Bread	
Brown bread	7
Whole-wheat bread	11.1
Multigrain bread, dark	12.9
Breakfast cereals	
Muesli, crunchy	9
Oat-flakes	12.8
Potatoes, cooked	2.3
Grain (rice, pasta)	
Brown rice, cooked	2.6
Rice, parboiled, cooked	2.8
Rice, peeled, cooked	3.1
Whole-wheat pasta, cooked	5.4
Vegetables, cooked	
Carrot	0.6
Leek	1
Cauliflower	2.3
Broccoli	3
Legume	
White bean, cooked	8
Lentils, cooked	8.2
Tofu	11.8
White bean, dried	20
Soybean, dried	35.9
Fruit	
Apple	0.3
Mandarin	0.9
Banana	1.1
Nuts and seeds	2.5-27.2
Cashew	18.2
Sesame seeds	20
Almonds	21.1

[µ] The protein content is based on Belgian food composition table (2013) [371]

2.2. Protein intakes

From a global perspective, inadequate protein intake is today still a major public health concern in many developing countries, while this is much less of concern in the Western world [122]. However, protein intake has been reported to be adequate in most Asian countries, including Japan [524], Vietnam [233] and China [434; 573].

Results from the National Health and Nutrition Examination Survey (NHANES) in US, 2007–2010, indicated that male and female adults consumed sufficient protein (98.6 and 67.0 g/d, respectively [389]. In Europe, a Spanish study reported mean total protein intakes of 97.4 g/d in men (69.6 g/d animal protein; contributing 18.9% to energy intake) and 79.0 g/d in women (56.8 g/d animal protein; contributing 19.4% to energy intake) [432].

The consumption of protein in Asia is still much lower than in Western countries. The ongoing China Nutrition and Health cohort study revealed that protein intake had decreased over time (83 g/d in 1989, 73.3 g/d in 2004), but the protein contribution to energy intake had increased gradually (11.5% in 1989 to 12.5% in 2004) [573]. One recent result from population- based health and nutrition survey in a relatively rich province (Zhejiang province), 2009 – 2011, shows that average protein intake was 71.1g/d for urban male residents [225]. Likewise, average protein intake in Japanese populations from the National Nutrition Survey was 78.1 - 96.8 g/d in men and 64.3 -78.2 g/d in women, stratified by age groups [524].

Evidence indicates that at least two thirds of total dietary protein was from animal sources including meat, fish, poultry and dairy products in Western standards [432;454;573], which is opposite to the protein composition in Asian standards. Half of dietary protein in some Asian diets was derived from plant products [326;524].

2.3. Protein intakes and body weight and body composition

Recent reviews show that HP diets might be beneficial for obtaining and maintaining more optimal body weight and body composition, and as such preventing OB related chronic diseases [186;272;426;530]. Observational studies reported that protein intake was inversely associated with body weight, and positively associated with building body composition including fat free mass, lean mass and appendicular lean mass of WC, hip circumference, biceps, triceps, subscapular [183;495]. In the Health, Aging, and Body Composition (Health ABC) Study, increasing protein intake was positively associated with weight loss over the 3-year follow-up, but also slightly with lean mass loss [218]. One recent 6-year follow-up longitudinal study conducted on 8–10-year-old children showed that moderately high HP could have influenced the development of body composition via decreasing body fat gain and increasing fat free mass gain during puberty in girls [495]. The finding depended on the combination of the specific amino acids arginine and lysine [495]. However, the representative population-based National FINRISK 2007 Study showed that the intake of protein was the lowest in the lean participants (25–74 years) and higher in normal-weight, obese and OW ones [321]. Most studies on dietary protein intake in relation to OB were intervention studies.

Intervention studies also suggested that a modest increase in consumption of dietary protein may impact not only weight loss and weight maintenance, but also the improvement of body composition i.e. an increased fat free mass) [159;164;183;301;340;423;455;476;529]. A 6-month randomized prospective study showed that 15 clinically healthy male volunteers on a HP diet (19.5% energy) had a larger decrease in body weight (-2 kg, $P<0.05$) in comparison to those on the NP (15.2% energy) (0.7 kg, $P>0.05$) [164]. Fat mass decreased more in the HP group, although no statistical significance was found between the two groups [164]. HP intake is believed to help individuals performing physical activity and as such to improving body mass by building lean body mass and reducing fat free mass. A modest increase in consumption of both dietary protein and fiber in OW women significantly reduced measures of adiposity, body mass (1.2 kg), total body fat (1.0 kg) and central body fat (0.7 kg) with no loss of lean mass during a 10-week dietary intervention [340]. 64 and 68 obese patients in a randomized trial received a low-carbohydrate/high-protein (LCHP) diet and a low-fat/high-carbohydrate (LFHC) diet, respectively [423]. After 6-month intervention, the subjects in the LCHP group lost significantly more weight (5.8 vs. 1.9 kg; $P = 0.002$), compared to those subjects in the LFHC groups. In addition, a high protein/low glycemic index diet was beneficial in maintaining body weight in OW European adults after weight loss [288] due to the inhibition of energy intake and satiating effects [447]. A protein-rich diet may have adverse health implications, which can affect kidney function because of high intake of nitrogen [263]. However, the protein sources can be a critical factor as plant protein may lower nonvolatile acid load and have decreased bioavailability of phosphorous [45].

Concerning the dietary sources of protein, the debate is still ongoing. Animal protein and plant protein have different amino acid composition and therefore different influences on weight and body composition, with more benefits of plant proteins than animal proteins on weight loss and optimal body composition as found in observational studies [80] and intervention studies [14;51;272;300]. This might be due to the fact that primary sources of animal protein are rich in saturated fat, cholesterol [223]. Dietary protein intake is essential for children's bone development in a critical period; however, extreme high intake from animal sources at early children's age was suggested to be associated with an unfavorable body weight and BMI, which is responsible for late OB risk [194]. Gunther *et al.* reported that children in the cohort typically experienced adiposity rebounded at the age of 5–6 years, a potentially critical period for OB development when weight gain started to regain importance compared with height gain [194]. In addition, plant protein intake was observed inversely associated with OW/OB in apparently healthy middle-aged American men across 7-year-follow up, but animal protein was positively associated [80]. Similar findings were reported that whey protein positively increased body weight in all the normal-weight and OW subjects participating in a cross-sectional clinical intervention study, but OW male adolescents had much less weight increase compared to normal-weight peers [51].

Allison *et al.* reported that soy-based meal replacement formula (90 g soy protein isolate and 8 g vegetables protein) resulted in reducing significantly body weight (-7.0 kg vs. -2.9 kg), fat mass (-4.3 kg vs. -1.4 kg) and waist circumference (WC) (-6.0 cm vs. -2.9cm) among obese adults after 12 weeks compared to control group (18g lean meats protein, 10g vegetables protein, 15 g starches protein and 16 skimmed dairy protein) [14]. Likewise, randomized controlled clinical trials (RCT) concluded that high plant protein based diets, soy protein in particular, increased significantly weight loss among obese subjects [14;272;300]. However, no strong evidence from randomized trials proves the above hypothesis [18;34;35;445;562]. Some findings from several short-term RCTs suggest that animal protein can be beneficial for [18;164;272;301;320;331;459] body weight and body composition among obese subjects like plant protein. Average weight losses of fat and lean weight were found significantly in obese subjects on both casein and soy proteins after 16-week-intervention [18]. Randomizing a soy protein-based low-calorie group (45 g) and a traditional low-calorie group (30 g animal protein and 15 g soy protein) to obese adults with no history of chronic disease affected on reduction in body fat percentage (BF%) and WC [301], which may be due to the mechanism of energy restriction [320]. Two randomized trials found weight loss in diets rich in animal sources [164;331]. Generally, the negative relation between animal protein and BMI, and body composition is suggested to refer only to OW and obese individuals and does not affect healthy individuals with normal-weight and healthy BMI [51]. This can be explained by metabolic differences in OW and obese before and after controlling intakes of energy and fat and compared to normal-weight individuals [80].

2.4. Protein intakes and blood pressure

The majority of studies conducted on hypertensive patients determining the effects of dietary protein intake on BP suggested that a moderate increase in protein intake will lower BP [200;215;351;563]. A study conducted on 100 obese patients with MetS randomly assigned to either a diet relatively rich in carbohydrate and low in protein or a diet that was low in carbohydrate and high in protein [351]. The results of this study showed an inverse relationship between BP and dietary protein intake [351] because the substitution by protein can improve endothelial function with vasodilation [295] and in diuresis [91]. Similar results were found in previous population-based observational studies [443;461]. Liu *et al.* conducted a meta-analysis that demonstrated inverse associations between dietary protein intake and systolic blood pressure (SBP) and diastolic blood pressure (DBP) from 2 longitudinal studies and 9 out of 11 cross-sectional studies [307].

Many studies reported that the antihypertensive effect of protein is related not only to the amount of protein in the diet, but also to the source of protein. A recent study demonstrated that plant protein intake had more health benefits on BP than animal protein intake [149]. It was reported that plant protein was significantly inversely associated with BP, while no significance was found with animal protein. Observational studies and clinical trials suggest that HP intake, protein derived from plant sources in particular, may reduce BP, due to the role of the amino acid arginine affecting on a vasodilator through nitric oxide [522]. The Nutrition and Health of Aging Population in China cohort study showed weak inverse associations between soy protein intake and elevated BP in men (P-trend =0.049) [381]. Soy protein has been proven beneficial on BP among those living in the Western counties compared to Asian countries. The potential effect is due to a high arginine content and antioxidant activity [498]. Dietary intervention with soy protein on adults reported that soy protein resulted in a great decrease in BP compared to non-soy protein [200;527]. Randomizing the soy protein-based low-calorie group (45 g soy protein) and the traditional low-calorie group (30g animal protein and 15 g soy protein) diet to obese subjects, the soy protein-based low-calorie group had a greater effect on reducing BF%, TC and LDL-C concentration after an eight-week intervention compared to traditional low-calorie group [301]. Azadbakht *et al.* reported that three intervention diets on postmenopausal women with MetS in a randomized crossover clinical trial, including red meat- Dietary Approaches to Stop Hypertension (DASH) diet, soy-nut-DASH diet and soy-protein-DASH diet, had non-significant effects on SBP and DBP [35]. The none significant impact of soy protein intake on BP might be due to soy-protein containing isoflavones, which may be needed in high quantities of soy protein to affect BP [179;244]. Thus, Sagara *et al.* investigated the effects of dietary intake of soy protein and isoflavoneson on CVD in high risk middle-aged Scottish men by assigning at least 20 g of soy protein and 80 mg of isoflavones, or a placebo diet for five weeks [419]. This study found a significant decrease in both SBP and DBP among men consuming soy protein and isoflavones. Most studies have examined the association between soy protein and BP, but few were on vegetables and fruits. In a clinical trial of DASH, a diet rich in fruits and vegetables substantially lowered BP [23]. The HP diet (contributing 18% to energy intake) which was rich in fruits, vegetables, and low-fat dairy foods reduced SBP and DBP of adults after two weeks and sustained for six more weeks due to the reduced intakes of saturated and total fat [23].

2.5. Protein intakes and blood lipid profiles

HP intakes have not only been associated with weight loss and body composition including fat loss alongside improving lean mass retention, but have also been associated with a beneficial effect on plasma lipids in comparison with high carbohydrate diets, probably due to the increased satiating effect of protein with the modest reduction in carbohydrate [212;276]. On one hand a recent systematic review of RCTs demonstrated that LCHP was significantly effective on improvement in triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) (weighted mean difference: -0.17 mmol/ L and 0.04 mmol/L, respectively) at 6 months compared to a low-fat/low-calorie diet (LFLC) [212]. Quality evidence derived from one systematic review and meta-analysis of 74 RCTs, on the other hand, shows that HP diets had non-significant effects on blood lipid profile including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and C-reactive protein (CRP). However, a small positive effect on HDL-C concentration was observed [426]. In a crossover study, subjects with moderate hypercholesterolemia were randomly assigned to either LCHP or low-protein/ high-carbohydrate diet (LPHC) for 4–5 weeks [537]. Substitution of protein for carbohydrate with restriction of dietary fat and cholesterol significantly lowered plasma TC, LDL-C, TG and very low-density lipoprotein cholesterol (VLDL-C)-TG with increasing HDL-C concentration among those subjects [537]. A relatively high protein and high fiber (HPHF) intake lowered TC and LDL-C significantly among OW or obese women after 10-week intervention [340].

One four-week randomized crossover study was conducted on twenty hyperlipidemic subjects, aged 35-71 years, with a HP diet (specifically wheat gluten; 27% energy from protein) and control diet (16% energy protein) and found significant decrease in TG and oxidized LDL-C in the higher protein diet [246]. More studies were performed on soy-based diets as soy-protein diet is considered as effective in treating OB, T2D and CVD. Evidence shows that high soy protein intakes improve blood lipid profiles in adults in comparison with animal derived-proteins [301;445]. Soy-protein-rich foods were provided to OW women, aged 25 to 49 years, and resulted in decreasing plasma TG levels [459]. Li *et al.* conducted a 12-months dietary intervention on obese subjects (≥ 30 years) and reported that a soy-based diet (15 g soy protein) made a significant reduction in TG and hs-CRP at 6 months and 12 months, and TC at 6 months only [300].

It is known that animal protein diets may increase the consumption of energy, total fat, saturated fat and cholesterol intake. HP diet (20% energy protein, more sources: beef, pork, ham and poultry) was reported not to improve blood lipids among healthy men than normal protein diet (15% energy protein) [164]. The shift from the typical traditional Chinese dietary pattern and lifestyle to the Western dietary pattern and lifestyle has caused the Chinese nutrition and lifestyle transition. Findings of Chinese Nutrition and Health Survey, conducted in children (6-13 years) from Beijing and four provincial capital cities indicated that the Western dietary pattern rich in proteins from animal sources was positively associated TG and LDL-C levels, but inversely with HDL-C [434]. However, animal sources-derived protein diets made improvement in blood lipid profiles among OW subjects [164;331]. Melanson *et al.* conducting a RCT on OW women concluded that the beef-consumption or chicken-consumption dietary group resulted in significant reductions in blood TC and LDL-C [331]. The quality of protein sources can be playing a role due to less fat intake and fat mass loss [331].

2.6. Protein intakes and glycemic or insulin response

HP intake is associated with effects on glucose metabolism in large population-based studies by increased stimulation of insulin into the blood stream [306] due to an effect on glucose metabolism directly (substrate-mediated) and indirectly (hormone-mediated) by increasing availability of amino acids in liver [275]. One systematic review collecting 74 RCTs indicates that HP diets had insignificant effects on fasting blood glucose in comparison with low protein diets [426]. Recent short-term interventions based on LCHP such as Atkins diets were reported to have a favorable impact on weight loss and blood glucose hemostasis [403;482]. In addition, HP was suggested to be associated with improvement in overall glucose control among untreated T2D, via lowering postprandial blood-glucose concentrations, and glycated hemoglobin decreased significantly compared to a conventional high-carbohydrate diet [177].

More concerns are related to the impact of protein sources. Epidemiological studies have suggested that high total protein intakes, especially from animal sources, will increase the risk of T2D in middle-aged individuals [452;457]. Sites *et al.*, concluded from a RCT on postmenopausal obese women that soy protein and an isocaloric casein placebo for 3 months made inverse and positive changes in fasting insulin (-1.1 vs. 1.9) and fasting glucose (-0.33 vs. 1.80), respectively [445]. A recent RCT suggested that vegetable protein, soy-protein in particular, had an inverse impact on plasma glucose concentration, insulin response among pre-obese and obese subjects [272;300;459]. Soy-protein-rich diet had a significant effect on plasma glucose and of time on plasma insulin [459]. Li *et al.* conducted dietary intervention on OW and obese subjects and found that fasting plasma glucose was significantly reduced (126.4 mg/dl) and hemoglobin Alc levels improved by 0.49% in a soy-based meal group at 6 months compared with an individualized diet group [300].

High animal protein intake was suggested to have similar inverse impact on blood glucose and insulin among obese subjects [18]. A parallel dietary intervention conducted on OW and obese volunteers showed that a high-protein diet of meat, poultry, and dairy foods (HP diet: 27% of energy as protein, 44% as carbohydrate, and 29% as fat) decreased significantly more glycemic response among OW volunteers than a standard-protein diet (SP diet: 16% of energy as protein, 57% as carbohydrate, and 27% as fat) [159].

2.7. Mechanisms

Protein intake, containing at least 10-15% of energy, can increase satiety, decrease subsequent energy intake and decrease total fat and cholesterol intake, which can reduce appetite and increase energy expenditure during periods of excess availability of foods. The mechanisms which relate animal and plant protein intakes with OB and chronic diseases are unclear. One proposed mechanism is that animal proteins derived from beef, pork, and poultry provide an important amount of energy, and are positively associated with cholesterol and saturated fatty acid intakes, which may result in increased risk of OW and OB, and their consequences. The intake of plant proteins, conversely, was considered to be an important factor to control body weight and improve body composition, BP, and blood lipid profiles because of their associations with lower intakes of energy, total fat cholesterol, and saturated fatty acids, and higher polyunsaturated over saturated fatty acid ratios [326].

In addition, dietary protein triggers release and promotion of both insulin and glucagon. Dietary protein and amino acids (arginine, cysteine, glutamate, glutathione, leucine, taurine and tryptophan) are involved in glucose metabolism via stimulation of insulin and glucagon secretion and by serving as substrates for gluconeogenesis [403]. (Figure 1.1 and Figure 1.2). The protein content of diets will preferentially support glucagon activity and possibly decrease IGF-I synthesis due to relatively low essential amino acid content of some plant proteins. Plant protein based diets low in fat, and rich in fibers, decreased propensity to OB – will promote good peripheral insulin sensitivity and thus down regulate insulin secretion [326].

Furthermore, although a potential beneficial effect of protein on BP has not yet been clear, several hypotheses have been proposed (Figure 1.3). First, dietary protein is related to synthesis of cellular ion channels, influencing BP regulation indirectly because HP intake may induce natriuresis, leading to lower BP. Second, dietary protein and some amino acids were shown to improve insulin sensitivity and glucose metabolism, thus, regulating BP [498]. Third, dietary protein may result in a higher concentration of the amino acids tyrosine and tryptophan in the blood vessel wall, triggering a vasodilatory response because of improvement of oxidativestress, reduction of vascular intracellular calcium and increase in nitricoxide production.

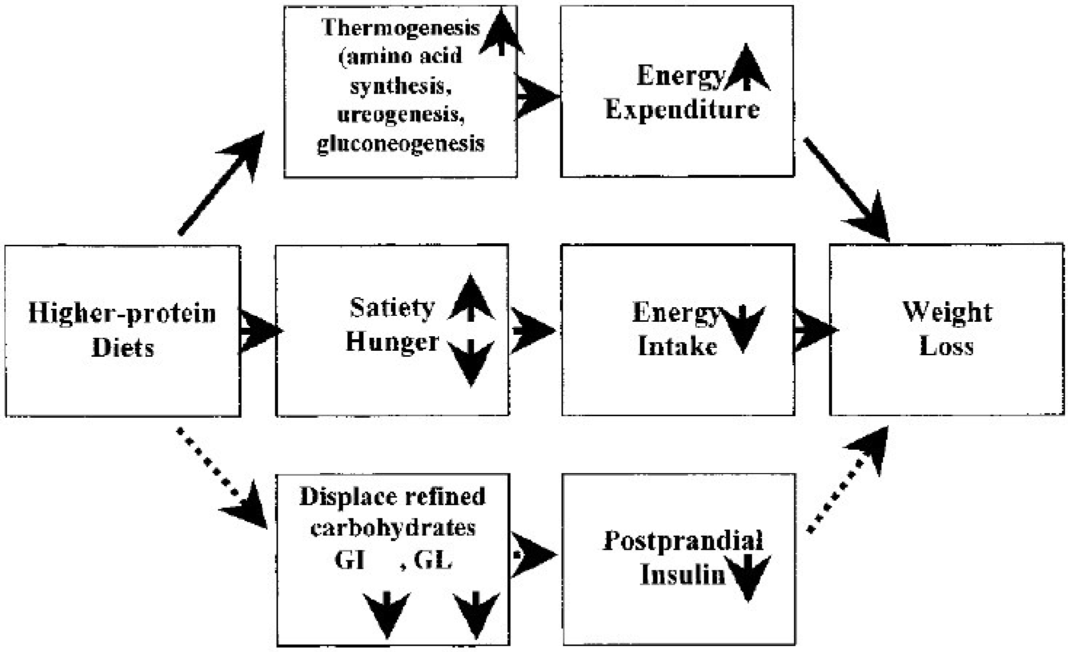

FIGURE 1.1. The mechanism of dietary protein affecting weight loss [223].

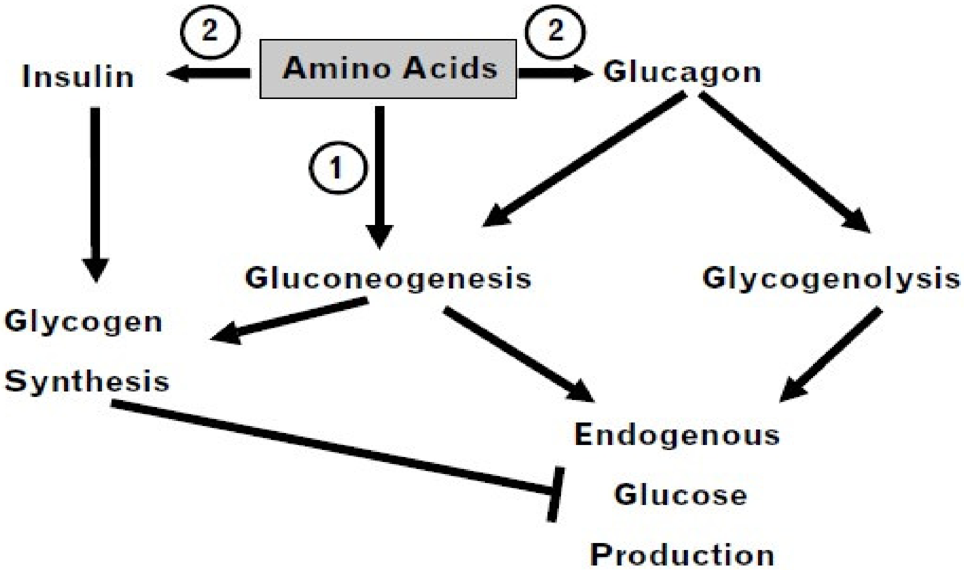

FIGURE 1.2. Potential direct (1) and indirect (2) effects of amino acids on blood glucose and insulin metabolism [403]

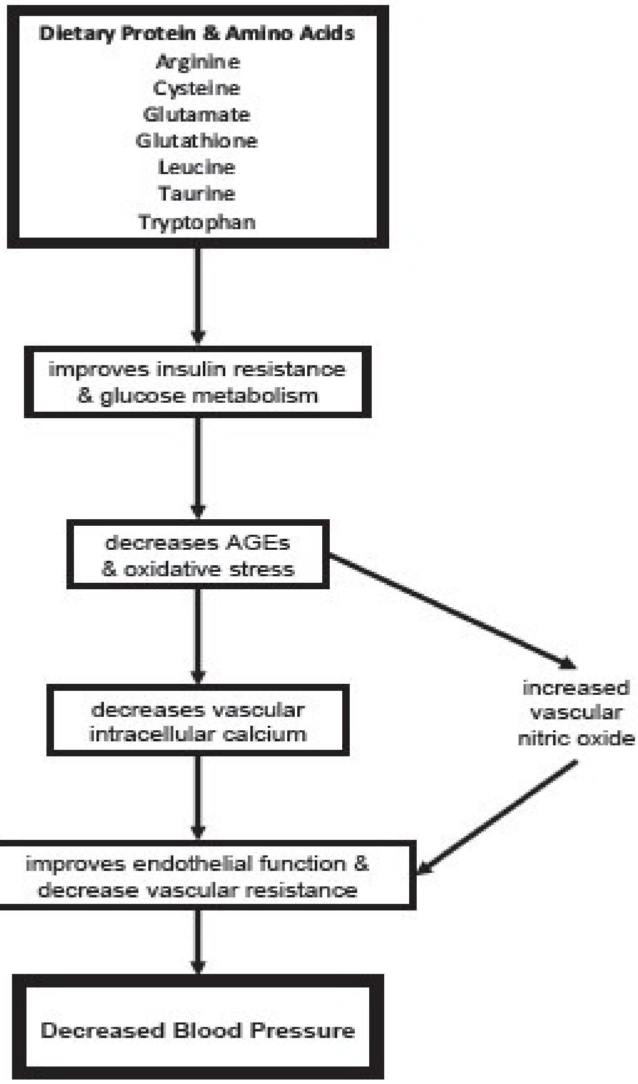

FIGURE 1.3. The antihypertensive mechanism of dietary protein and amino acids [498]

2.8. General summary of protein intake

Protein intake was adequate not only in the developed countries, but also in many developing countries based on the recommendation (contributing 10-15% to the total energy intake) proposed by WHO. Regarding the contribution of food sources to total protein intake, evidence showed that two thirds of total dietary protein in the Western countries was from animal sources, whereas 50% of total protein intake in the Asian diets was from plant foods.

Observational, intervention studies and RCTs show that consumption rich in protein may prevent OB and its related consequences in both normal and obese individuals via decreasing body weight, building body composition (lean mass and fat mass), reducing BP (SBP and DBP), improving blood lipids (TC,TG,LDL-C, HDL-C and CRP), and regulating fasting glucose and insulin.

Concerning the dietary sources of protein intake, plant protein intake can benefit more on prevention of OB than animal protein intake. However, animal protein intake may be inversely associated with/ impact on the indicators of OB and its related consequences only among OW and obese individuals.

3. DIETARY FIBER

3.1. Definitions and dietary sources

DF is the edible parts of plants and resistant starch, which are not hydrolyzed by endogenous enzymes in the human small intestine with complete or partial fermentation in the large intestine, to provide potential health effects [15]. DF is defined by the Codex Committee on Nutrition and Foods for Special Dietary Uses as carbohydrate polymers with ten or more monomeric units [115]. However, DF includes non-digestible carbohydrates with three to nine monomeric units (degree of polymerizations) in the definition of Europe and China [220;336]. DF can be divided into WSF from the sources of legumes, oats, fruits, vegetables, root tubers and root vegetables, and WIF from the source of whole grain foods, wheat and corn, nuts and seeds, potato skins, lignans, and the skins of some fruits (Table 1.2). The terminology of soluble fiber indicates to dissolve in water forming viscous gels, while insoluble fiber does not dissolve in water of the human's gastrointestinal tract. DF is strongly recommended by the WHO (20 g/d non-starch polysaccharides (NSP); >25 g/d total DF) to prevent OB and other chronic diseases [551]. Currently international recommendations for DF intake proposed by WHO [551], USDA [489], European food safety authority (EFSA) [158], Institute of Medicine (IOM) [234] and Belgian Superior Health Council (BSHC) [160] are related to age, gender and energy intake (Table 1.3).

Table 1.2. List of fiber contents (g fiber/ 100g) from main food sources

Food sources [µ]	Fiber (g/100g)
Bread	
White bread	1
Brown bread	5.7
Whole-wheat bread	6.4
Multigrain bread, dark	6.8
Breakfast cereals	
Cornflakes	2.5
Muesli, crunchy	7
Oat-flakes	8.5
Muesli with fine fruit	10.3
Potatoes, cooked	3.1
Grain (rice, pasta)	
Rice, peeled, cooked	0.5
Rice, parboiled, cooked	0.9
Brown rice, cooked	1.5
Whole wheat pasta, cooked	2.7
Vegetable	
Cucumber, raw	0.7
White cabbage, cooked	1.4
Chinese cabbage, raw	2.5
Leek, cooked	2.7
Carrot, cooked	2.8
Cauliflower, cooked	3
Legume	
Lentils, cooked	4.2
White bean, cooked	11.4
Soybean, dried	22
White bean, dried	28.6
Red bean, dried	28.6
Fruit	
Melon, Galia	0.6
Apple, peeled	1.5
Banana	1.7
Kiwi	3
Green olive	4
Raspberry	7.4
Nuts and seeds	
Cashew	2.9
Walnut	6.7
Hazelnut	8.2
Sesame seeds	11.2

[µ] The fiber content is based on Belgian food composition table (2013) [371]

Table 1.3. International recommendations of dietary fiber

Age group	Recommendation							
	WHO (g/d)	EFSA (g/d)	USDA		IOM (g/d)		BSHC (g/d)	
	Both genders	Both genders	Male	Female	Male	Female	Male	Female
1-3	>25 g/d	1-20	14 g/ 1000 kcal		19	19	15	15
4–8		10-20			25	25	25	20
9–13		15-33			31	26	40	30
14–18		15-33			38	26	30	30
19–50		15-30	38 g/d	25 g/d	38	25	30	30
>50		15-30	30 g/d	21 g/d	30	21	30	30

3.2. Fiber intake

Globally DF intake is not adequate. The NHANES, 2009 - 2010, reported that total DF intake in the US was 13.7 g/d in children and adolescents aged 2 to 19 years and 17.1 g/d in US adults [464]. The European level of DF intake was reported between 0.9 and 3.5 g dietary fiber/MJ in children [284], 14.0-26 g/d in male adolescents; 14.0-22 g/d in female adolescents; 18.0-26 g/d in male adults; 16.0-26 g/d in female adults15.0-31 g/d in elderly men, and 16.0-23 g/d in elderly women [150]. The Spanish national nutrition survey shows an intake of 18.7g/d DF in male adolescents, 17.4 g/d in female adolescents, 18.6-20.2 g/d in male adults and 15.4-18.1 in female adults [432]. In addition, similar results from The Third Italian National Food Consumption Survey, INRAN-SCAI 2005-2006, were reported [433]. The consumption of mean DF was 14.4 g/d in children aged 3 to 10 years, 18.1 in male adolescents and 16.4 g/d in female adolescents, 19.6 g/d in male adults and 17.7 in female adults aged 18 to 50 years, and 21.6 g/d in male old generation and 18.7 g/d in female old generation aged 65 years and above [433]. While, the level of DF intake in Asian countries decreased significantly due to the nutrition transition influenced by a Westernized negative lifestyle [519], DF intakes were reported 18.8 g/d for males and 18.0 g/d for females in Japanese ≥ 30 years [173], and 15.7 - 17.6 g/d for male and 13.5 - 16.4 g/d for females in Chinese aged 18-45 years [519].

Most health advisory groups provide guidance for increasing DF intake to obtain the recommended levels of DF consumption from foods, especially fruits, vegetables, and whole grain products [158;489;551].

3.3. Fiber intake and body weight and body composition

Previous studies have been conducted to evaluate the impact of DF intakes on OB including observational and intervention studies. Most cross-sectional studies [52;53;119;121;348;463;493;566], longitudinal studies [140;270;277;308;372], and intervention studies [61;156;216;332;340;479;501] have concluded that DF assists in weight loss, lower BMI and body composition, including WC, BF%, subscapular skinfold thickness and triceps skinfolds, in children, adolescents and adults of Asian and Western populations. OW and OB subjects were observed consuming significantly lower DF intakes than non-obese subjects [53;119]. Lower DF intake was observed to be associated with a greater risk of OW in teenage students [121]. Similar results of DF intake were reported to be inversely associated with body weight and BMI independently of gender [277;493], as well as BF% [119]. A 10-g/d higher total DF intake was responsible for 39 g/year weight loss and 0.08 cm/y WC reduction after 6.5 years follow-up among European adults aged 20-78 years [140]. Lupin-derived WIF had the net effects on body weight (-0.4 kg), fat mass (-0.5 kg) and BF% (-0.5 kg) in OW and obese Western Australian male adults after 16-weeks randomized trial [216]. However, intervention studies have investigated whether DF intake assists in weight loss and concluded that a low-calorie DF diet significantly improved weight loss (8.0 kg) with the placebo group losing 5.8 kg after 24-week treatment [64]. Weight loss, especially around the waist, was found in the intervention group [61]. Melanson *et al.* found similar weight loss in both intervention groups, hypocaloric with fiber-rich diet plus exercise and the hypocaloric diet plus exercise, among OW and obese adults [332].

The role of DF is to decrease body weight or attenuate weight gain and build body composition due to WSF and WIF. WIF derived from cereals and legumes [140;283;308;348;372;493] and WSF derived from vegetables, and fruits [58;348;367] was associated with lower body weight, BMI, WC and BF%. DF may significantly decrease energy and dietary fat intakes. Samra *et al.* concluded that 33 g cereal- derived WIF reduced appetite and lowered food intake [424]. Fruits and vegetables might play a role in weight management and etiology of OB, probably because of reduction of other energy/fat-dense foods [119]. A “high-fiber diet” enriched in vegetables, whole-grain cereals and legumes was negatively correlated with OB indices, including BMI, WC and triceps skinfold in girls (mean age: 11 year) [566]. The same amount of DF (10 g/d) DF derived from cereals, vegetables and fruits were found to be responsible for weight loss (-77 g/year) and weight gain (2 g/year) among European adults [140].

Conversely, one longitudinal study and one randomized controlled double-blind parallel trial contradicted the above conclusion on the impact of DF [52;235]. The findings of cross-sectional data derived from the 1991 China Health and Nutrition Surveys indicated that DF intake was strongly and positively associated with OW status in 5783 Chinese adults (aged 20 -59 y) [463]. Fruit-derived fiber was reported to be positively associated with BMI in Chinese patients diagnosed with T2D [93]. Lupin-derived DF intake had effect on weight loss and fat mass in a short-term study [270], but not in long-term studies [52].

3.4. Fiber intake and blood pressure

Increasing consumption of DF is often accompanied by a reduction in SBP and DBP. Therefore, the Western dietary pattern may be associated with an increased risk of hypertension compared to the traditional Asian dietary pattern [434;439]. SBP and DBP were observed to be significantly higher among Chinese children with a Western dietary pattern which is lower in DF [434]. Observational studies have suggested that DF intake is inversely related to BP [25;26;434;439]. Consistently, large prospective studies have reported that increased DF intake was associated with lower risk of hypertension and lower BP [25;26]. Earlier clinical trials proposed the hypothesis that high DF diets had effects of lower BP and impact on moderation of BP [16;132;280;492]. A meta-analysis of RCT supported the above hypothesis of an inverse relationship between DF intake and BP [531]. The finding indicated that increased intakes of DF may result in a reduction of BP among hypertensive patients receiving at least 8-week intervention [531]. Recent RCTs have shown that a significant reduction in BP (both SBP and DBP) during consumption of a high fiber diet [81;156;202;245;247;395;422]. One recent 3-month RCT conducted on 772 elderly with high risk of T2D or coronary artery disease assigned a low-fat diet or two Mediterranean-style diets were reported that the higher the increment of DF intake, the greater the reduction in SBP and DBP was observed after 3-month intervention ($P<0.001$, $P=0.002$, respectively) [156]. Although some early RCTs showed the opposite results, an increase in DF is strongly recommended by the WHO because DF treatment is considered as a safe and practical approach to reduce cardiovascular risk in hypertensive populations [551].

The efficacy of WSF [81;245;247;422] and WIF from different food sources on BP has been investigated [52;283;422]. In a parallel-design trial, participants randomly assigned to consume lupin-enriched foods or matching high-carbohydrate control foods showed that the effects of increasing legume-derived DF intake on BP after 12-month intervention have been reported as a modest-to-moderate reduction in SBP and DBP [395], which is in line with the finding from another 8-week oat intervention study [422]. Similarly findings of diets rich in vegetable and fruit fiber from observational studies were reported [198;435]. Fruits and vegetables are rich in potassium and this can result in increasing potassium intakes. A DASH-style healthy diet rich in fruit and vegetables, and low in salt, fat, and processed foods is recommended to the population health as it may impact on reduction of BP at a population level [198]. Likewise, a longitudinal investigation in healthy children and adolescents showed that higher fruit and vegetable intakes were associated with a 0.4 mmHg lower BP value [435].

3.5. Fiber intake and blood lipid profiles

Evidence shows that total fiber, WSF and WIF can improve blood lipid profile [53;77;277;375]. DF intake showed inverse correlation with plasma TC and TG and positive correlation with HDL-C [53]. Higher DF and WSF were suggested to reduce TC and LDL-C among healthy premenopausal women. Western dietary patterns rich in meat and meat products, and fat while low in DF from vegetables and fruits were positively associated with concentrations of LDL-C and TG (P<0.001 both), but inversely with HDL-C (P=0.0082) in Chinese children [434].

Leptin concentrations have been shown to be positively associated with the risk of OB and CVD, because it plays a role in fat metabolism and correlates with insulin resistance and other markers of MetS, independent of total adiposity [513]. DF was observed to be inversely associated with plasma leptin concentration (β=0.044, P=0.036) in Japanese population. Additionally, the higher intake of the fruit- and vegetable- derived DF was, the correlation was inversely associated with lower hs-CRP levels in men only [375].

Experimental studies also have shown therapeutic benefits of DF on blood lipid profiles, a risk factor for atherosclerosis and CHD [61;156;367]. Reduction in TC levels and increments in HDL-C were found highest among the Spanish elderly, who had either T2D or three or more of the CHD risk factors, in the upper 20% of DF intake (P=0.040 and P=0.020, respectively), and plasma concentrations of CRP were reported to decrease in parallel with increasing DF intakes (P=0.040) [156]. Significant reductions in LDL-C were also observed among participants with the greatest increases in WSF intake [156]. Similar studies reported that blood lipid profile was improved by WSF among obese subjects [367] and fruit-derived fiber was found not significantly correlated with LDL-C and HDL-C in Chinese patients with T2D [93].

3.6. Fiber intake and glycemic or insulin response

High levels of DF intake are associated with a significant reduction in the prevalence of diabetes based on estimates from prospective epidemiological cohort studies [335]. Epidemiological studies indicate that higher levels of DF intake play a significant protective role with respect to diabetes. A recent cross-sectional study has shown that total DF intake was correlated with glycaemia (r =–0.557, $P < 0.0001$) [53].

Short-term studies indicate that DF intake decreases postprandial glycaemia and insulinemia and enhances insulin sensitivity [424;525]. Fasting insulin sensitivity was significantly improved in long-term RCTs [52;61;156], but not in a short-term RCT [216]. Postprandial blood glucose concentration did not increase after consumption of 33 g/d cereals-derived WIF [424]. After one year dietary intervention by assigning lupin-enriched foods or matching high-carbohydrate control foods, an RCT showed significant reductions in fasting serum glucose values (mean net changes: -0.05 mmol/l at 4 months and -0.03 mmol/l at 8 months) and fasting serum insulin values (mean net changes: -1.19 mU/l at 4 months, -1.27 mU/l at 12 months) [52]. Likewise, two studies reported significant reductions in fasting serum insulin values in the dietary intervention groups assigned to low fat, high whole grains and fruits and vegetables (mean net changes: -2.26 mU/l at 4 months, -7.10 pmol/L, respectively) [61], compared to the control group given pamphlets containing minimal information on healthy eating. Insulin sensitivity was reported to be significantly improved in one study [52]. Hence, studies indicate that moderate increases in DF intake from foods or supplements are associated with a significant reduction in fasting serum glucose and insulin values and improved insulin sensitivity.

3.7. Mechanisms

High energy density diets increase food consumption when compared with diets with lower energy density. Diets rich in DF may promote a decrease in weight due to low energy density and displacement for energy or calories [119]. WSF can delay small bowel absorption, which may reduce cholesterol absorption [471]. The colonic fermentation of WSF results in the production of gases and short-chain fatty acids [474], which increases satiety through prolonging the intestinal phase of nutrient digestion and absorption [449]. Additionally, gases and short-chain fatty acids due to fermentation of WSF [474] can lower the glycemic index of foods, consequently, attenuating the insulin response [52;156]. Therefore, DF has been shown to enhance insulin sensitivity and improve vascular endothelial function [52;156] (Figure 1.4).

Additionally, DF intake, especially WSF, may have an indirect favorable effect on BP by improving mineral absorption in the gastrointestinal system [108]. As mentioned above, WSF have been shown to reduce insulin resistance and insulin levels. A major pathogenic mechanism for the development of hypertension can be insulin resistance and its concomitant compensatory hyperinsulinemia.

Furthermore, due to its anti-nutritive properties and non-digestibility, WIF can increase the bulkiness of stool and faecal mass, thereby shortening the transit time.

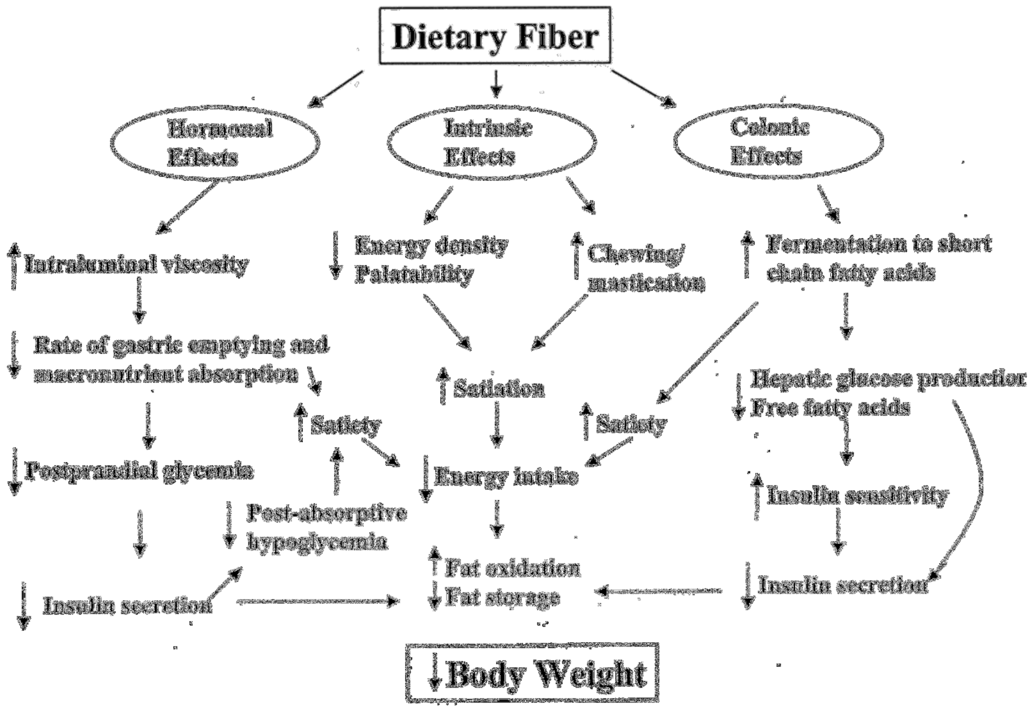

FIGURE 1.4. The mechanism of dietary fiber affecting physiologic measurements [392].

3.8. General summary of dietary fiber

Globally DF intake is inadequate, although it is strongly recommended to have a certain level intake. Due to the nutrition transition, DF intake was observed to decrease significantly in many Asian countries.

Results derived from observational and intervention studies prove that increasing consumption of DF may assist in prevention of OB, hypertension, hyperlipidemia, CVD, T2D and other related cancers among healthy, OB subjects and subjects who have high risk of CVD and T2D.

High consumption of DF from vegetables, fruits, cereals and legumes can promote a decrease in body weight and build body composition (e.g. WC and BF%.), reduce SBP and DBP, improve blood lipid profile, decrease postprandial glycaemia and insulinemia, and enhance insulin sensitivity due to lower energy density. However, some cross-sectional studies reported that DF from vegetables and fruits was positively associated with body weight, BMI and blood lipid profiles, which might be explained by the nature of study design.

4. SUMMARY ON STATE OF THE ART

Overall, dietary protein intake is adequate worldwide when looking at its energy supply, expressed as % energy, whereas DF intake globally is below the international guidelines/recommendations.

Evidence from observational and intervention studies, and RCTs indicates that high protein and high fiber intakes may protect against OW, OB and its related chronic diseases independently via lowering body weight, improving body composition, controlling BP, and improving blood lipid profiles, blood glucose concentrations and insulin response.

Considering the food sources of dietary protein intakes, findings based on the literature revealed that the consumption of plant protein better prevents against OB and chronic diseases in observational and intervention studies, and clinical trials, in comparison with animal protein. However, animal- derived protein intake has been inversely associated with the indicators of anthropometry and biomarkers in obese subjects.

Regarding DF, WSF and WIF from vegetables, fruits and legumes had impact on the lower risk of OB and its related diseases. Although some studies show the inconsistent results on DF from vegetables and fruits, a certain level intake of vegetables and fruits are still strongly recommended to improve health status and prevent OB and its related diseases. Fruits and vegetables are considered to play a role in weight management and etiology of OB and its related diseases, probably due to reduction of energy density.

More research is needed to disentangle the associations between these dietary components further.

5. THE OVERALL OBJECTIVES , TARGETS AND OUTLINE OF THIS THESIS

The overall objective of this thesis is to study and compare protein and fiber intakes in the traditional Chinese dietary pattern and the Western dietary pattern and to investigate if these two patterns are associated with OB and OB-related consequences. Chinese populations are known to consume more plant based diets rich in plant protein and fibers compared to European populations, which might be related to lower prevalence of OW and OB in Chinese populations.

Data from four different food consumption surveys were used to investigate the above mentioned **objectives**: (1) the Belgian national food consumption survey (BNFCS) (2004), (2) the Flanders preschool dietary survey (FPDS) (2002-2003), (3) the healthy lifestyle in Europe by nutrition in adolescence - cross-sectional study (HELENA-CSS) (2006-2007) and (4) the China health and nutrition surveys (CHNS) (2004). These surveys studied the following target populations 1) Belgian population aged ≥ 15 years; 2) Flemish (Belgian) preschoolers aged 2.5 to 6.5 years; 3) European adolescents aged 12.5-17.5 years; and 4) Chinese population aged ≥ 3 years.

The current dietary pattern can be different from the original traditional dietary pattern due to the nutrition transition (and as data were collected already in 2004). In order to gain more insight into the differences of the traditional Chinese dietary pattern and the Western dietary pattern and the associations between intakes of protein/fiber and the indicators based on 2 dietary patterns, dietary protein and fiber from main food groups were investigated. **<u>The primary objective</u>** of this thesis is to evaluate the consumptions of total dietary protein and fiber in four independent populations with different eating patterns and cultures, and to determine the main contributors to total dietary protein and DF intakes. Based on literature, diets rich in protein and fiber can have benefits on prevention of OB and its related chronic diseases. Concerning the dietary sources of protein and fiber, dietary proteins derived from plant sources were reported to be more beneficial and to impact on body weight, body composition, BP, blood lipid and insulin response than dietary proteins derived from animal sources. However, some studies didn't agree the above conclusion that animal protein had positive impact on OB and its co-morbidity. While evidence shows positive effects of total fiber WSF and WIF consumption on OB and its related chronic diseases WSF was reported to have an indirect impact. Therefore, **the second objective** of this thesis is to examine the associations between the consumptions of total dietary protein and DF, (and their main contributors), and indicators of OB and chronic diseases including anthropometric measurements in Belgian population, European adolescents and Chinese population and biomarkers in European adolescents. Because valid anthropometric data were lacking in the survey among Flemish preschoolers, the association with anthropometric data was not investigated in this population group. **The third objective** is to investigate the associations between intakes of animal, plant proteins and fiber, and the factors of SES and lifestyle in Flemish preschoolers.

As four research projects are included in this thesis, the data derived from four independent surveys were estimated based on standard national/ international food consumption tables,. Each project followed its own standard sampling, study design and methodology, which have been presented in *Chapter 2*. All above purposes (questions) are presented in four chapters (*Chapter 3*, *Chapter 4*, *Chapter 5* and *Chapter 6*).

The objectives **of the primary objective** are:

- To estimate the intake of animal and plant proteins, and DF in a representative sample of Flemish preschoolers, the Belgian population (≥ 15 years), European adolescents and the Chinese population (*Chapter 3*(*3.1.and 3.2*), *Chapter 4* (*4.1. and 4.2.*), *Chapter 5* (*5.1. and 5.2.*) and *Chapter 6*, *Appendix 3*);

- To identify the main contributors to animal and plant proteins, and fiber intakes in Flemish preschoolers, the Belgian population (≥ 15 years), European adolescents and the Chinese population (*Chapter 3* (*3.1.and 3.2*), *Chapter 4* (*4.1.and 4.2*), *Chapter 5* (*5.1.and 5.2*), *Appendix 4, Appendix 5, Appendix 6, Appendix8, Appendix 14 and Appendix 15*).

The objectives of **the second objective** are:

- To examine associations of dietary animal, plant proteins and DF intakes/ the intakes of main contributors, with OW and OB measured by BMI and WC (*Chapter 4* (*4.1. and 4.2.*), and *Chapter 6*);

- To examine associations of dietary animal, plant proteins and DF intakes, with the indicators of OW, OB and cardio-metabolic indicators including anthropometry and biomarkers (*Chapter 5* (*5.1. and 5.2.*)).

The objectives of **the third objective** are:

- To examine associations between the main contributors of animal, plant proteins and DF intakes, and f SES and lifestyle factors (*Chapter 3* (*3.1.and 3.2*))

In the general discussion (*Chapter 7*), the evaluation of the intake levels of protein and DF, and the comparisons between Chinese and Western intakes have been discussed in depth. In addition, referring to the second purpose, the comparisons of the findings of association studies performed in the Chinese and Western populations are being discussed and presented in this Chapter. At last, based upon the three purposes, dietary recommendations for improving diet and health status among all the populations are drafted.

In the end, the general conclusion of this thesis is presented in *Chapter 8.*

All the result chapters (Chapter 3, Chapter 4, Chapter 5) are based on a collection of scientific articles that are published or to be published in peer-reviewed journals.

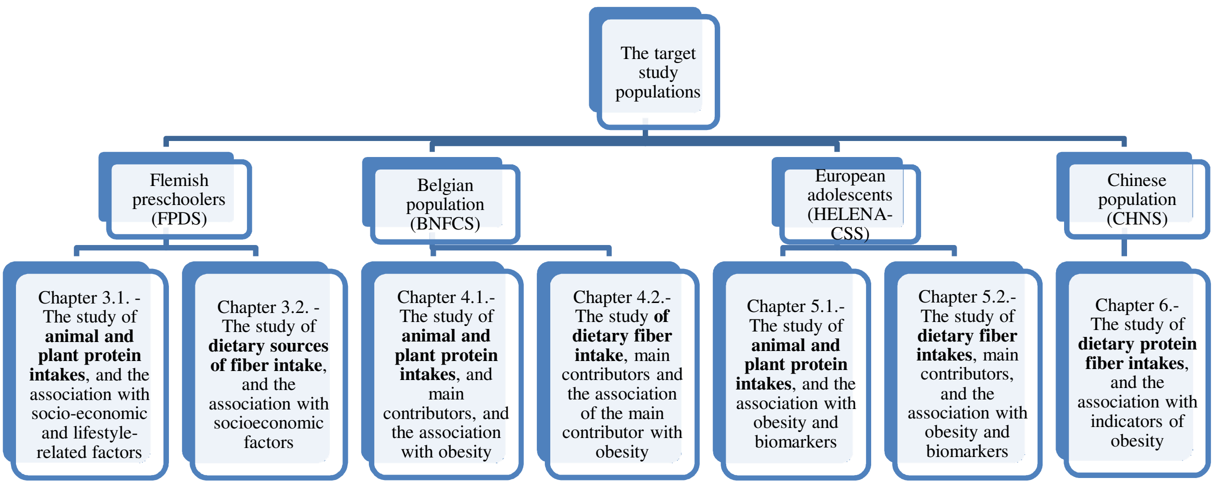

FIGURE 1.5. General view of outline

CHAPTER 2

METHODOLOGY: DESIGN, POPULATION, DIETARY ASSESSMENT AND MEASUREMENTS

1. STUDY POPULATION AND SAMPLING PROCEDURES

The time periods of the studies in this thesis indicate that the results can not reflect the current Chinese dietary pattern and nutrition status as the nutrition transition is ongoing in China. However, the dietary pattern and nutrition status in the European population are currently very stable and similar as dietary intakes a decade ago.

This thesis investigated four independent target populations covering all age groups, including preschoolers, young school-aged children, adolescents, adults and elderly generation, and various cultures and dietary behaviors. All the projects used a multi-stage cluster sampling procedure to draw the study sample for data collection.

FPDS was conducted in Flanders, a widespread region in Belgium and ran from October 2002 until February 2003. Flemish preschoolers (2.5-6.5 years) from both genders were the target population, stratified by the predefined age groups (2.5-3.9 and 4.0-6.5 years) [229]. A total of 2095 out of 2604 included preschoolers were invited in the study.

Using a cross-sectional epidemiological design, the target samples of preschoolers were selected on the basis of random cluster sampling at the level of schools, stratified by province and age [229]. Two stages of the cluster sampling were carried out: (1) schools were selected as primary sampling units from lists made available by the Ministry of Flanders for Education; (2) classrooms were selected as secondary sampling units. Each class was randomly selected for each age group (secondary sampling unit) within every school participating in the study, including all the children within the selected classes as final sampling units.

Preschoolers were excluded from the study if they: (1) stayed in an institution (e.g. a hospital school) with the foods provided by the institution; (2) did not attend school during the whole period of the fieldwork; (3) they lived abroad; and (4) when neither of their parents spoke Dutch. Thirteen out of sixty-three eligible nursery schools refused to participate in this dietary survey and seven schools refused to distribute food diaries. The remaining forty-three schools spread over the different provinces in Flanders participated in the study.

2095 preschool children were invited to participate in the whole study. Their parents/proxies were asked to complete an EDR. 1052 children returned an EDR (50% of the contacted). 26

children's EDR needed to be excluded because of quality problems of EDR. So 1026 children's EDRs were found eligible to be included. 330 out of 1026 children didn't complete 3-d EDRs, which were excluded. 55 out of 696 children who completed 3 good quality EDRs were excluded from the final analysis because of missing information of gender and age. Therefore, finally, 661 3-d EDRs were maintained in the final data analysis (Figure 2.1).

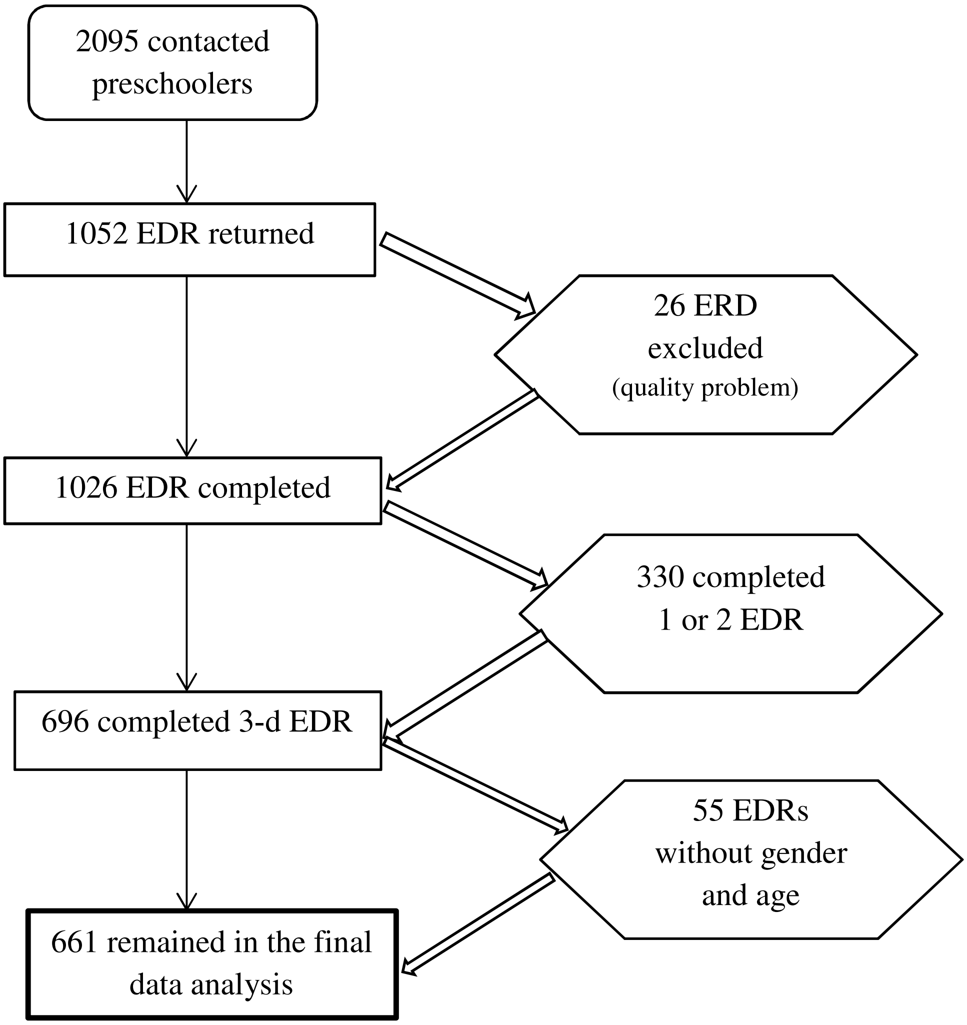

Figure 2.1. Participation and data selection of the Flanders preschool dietary survey

BNFCS [123] was performed in 2004 following largely the recommendations of the European Food Consumption Survey Method project (EFCOSUM) [79]. Belgian national citizens aged 15 years or older, residing in private households in Belgium, were eligible to participate in the national survey [123]. The population was stratified by sex and in four age groups (15–18, 19–59, 60–74 and ≥75 years). Approximately 400 individuals were allocated in each sex–age group. Institutionalized individuals, not able to speak one of the national languages or physically or mentally unable to be interviewed, were excluded from the survey.

A multi-stage sampling procedure was used to draw the study sample [123]. In the 1st stage, the data of the 10 provinces and the Brussels metropolitan area was obtained by stratification at the level of provinces. The Belgian municipalities which are the sixty-four primary sampling units were defined within each province during the 2nd stage. By ordering and systematic sampling, the municipalities were then stratified and only one municipality was chosen. In addition, large cities were selected more than once according to their multiple systematic sampling steps. In the end, almost 50 individuals were selected from each primary sampling units. In total, 9255 individuals were invited by a letter with an information brochure. 952 individuals could not be contacted and 760 individuals were not eligible to participate in the study. Out of 7543 eligible individuals, 3164 individuals completed at least 1 interview. Finally, 3083 participants with 2 completed 24-h dietary recall interviews were included in the final analysis (Figure 2.2.).

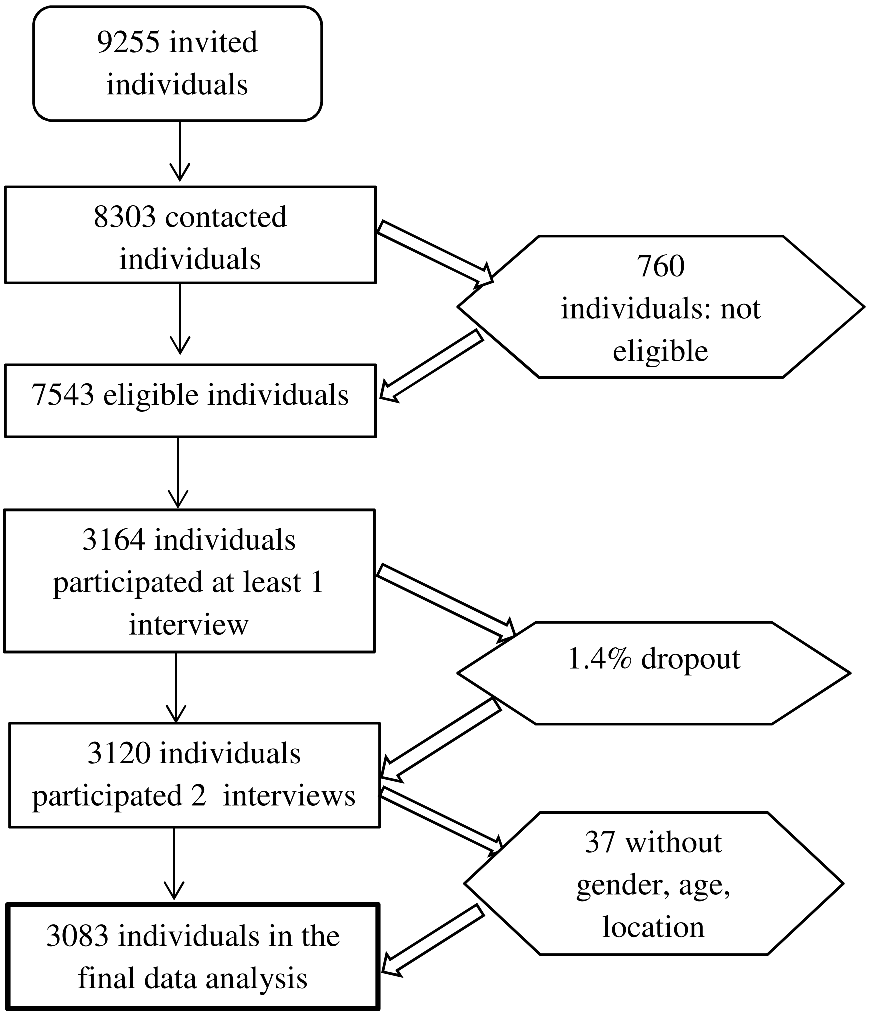

Figure 2.2. Participation and data selection of the Belgian national food consumption survey

HELENA-CSS is the first study providing data on the nutrition and main determinants of food choices and preferences among male and female European adolescents. HELENA–CSS in this thesis was carried out between October 2006 and December 2007. Male and female adolescents, aged 12.5-17.5 years, from ten European centers including Sweden (Stockholm), Greece (Athens and Heraklion), Italy (Rome), Pecs (Hungary), Sprain (Zaragoza), Belgium (Gent), France (Lille), Germany (Dortmund), and Austria (Vienna) were the target population in this thesis [341;342]. In the end, due to logistical reasons, adolescents from Heraklion and Pecs were excluded from the dietary intake analyses.

The average number of adolescents to be studied was estimated at 3300 (± 330 in each center). A multi-stage random cluster sampling procedure was used to select 3300 adolescents, stratified by geographical location, age and SES. Schools were randomly selected after stratification to guarantee diversity of the sample in cultural and SES strata [341;342]. 5759 adolescents were screened and contacted to participate in the study and a final sample of 3865 adolescents participated in the HELENA-CSS. 3582 eligible participating adolescents were included in the final data analysis. Male and female participants included in this study if they had provided complete anthropometrical measurements and completed >75% questionnaires including nutrition knowledge, eating behavior, food choice and preference and a questionnaire for parents. A total of 1804 out of 3528 adolescents from 8 centers completed all interviews and measurements used in this thesis and were included in the final analysis performed in this thesis (Figure 2.3.).

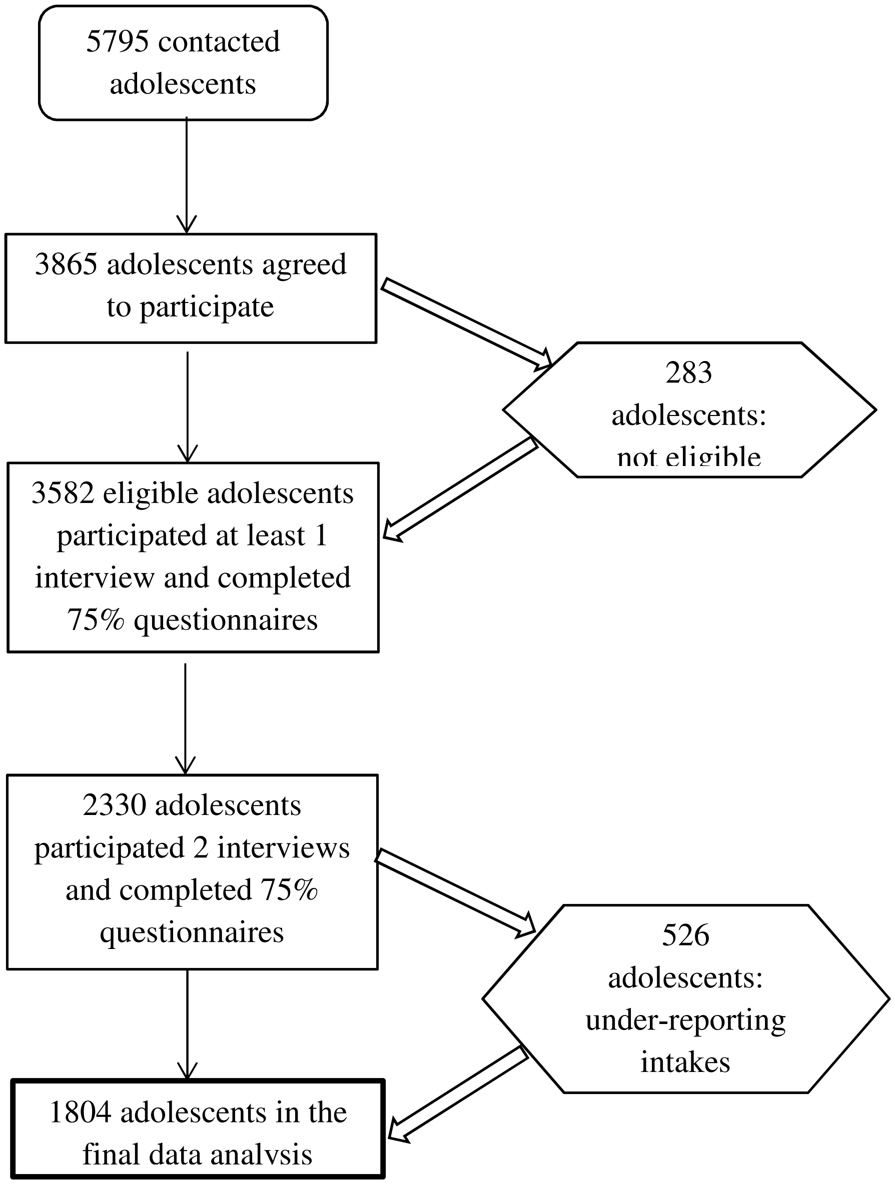

Figure 2.3. Participation and data selection in European adolescents participating HELENA study

CHNS, an ongoing cohort study, was designed to examine the effects of the health, nutrition, and family planning policies and programs implemented by national and local governments and to see how the social and economic transformation of Chinese society is affecting the health and nutritional status of its population. The impact on nutrition and health behaviors and outcomes is gauged by changes in community organizations and programs as well as by changes in sets of household and individual economic, demographic, and social factors. Data in this thesis are from a cross-sectional study carried out in 2004.

The estimated 16000 individuals (≥3 years) in both genders, from nine provinces (Guangxi, Guizhou, Heilongjiang, Henan, Hubei, Hunan, Jiangsu, Liaoning and Shandong) were considered to be included in this study [399]. The target sample was divided into three age groups: preschooler (3-6 years), school-aged children (7-17 years), adults (≥18 years) based on the Chinese dietary intake guidelines.

A multistage, random cluster sampling procedure was used to draw the sample collection in each of the provinces. Counties in the eight provinces were stratified by income (low, middle, and high) and four counties in each province were randomly selected by a weighted sampling scheme (one low income, two middle incomes, and one high income). In addition, urban areas initially excluding the county strata were later incorporated by including the provincial capital and a low income city from each province. Within each county, the township capital and three villages were selected randomly. Within each city, urban and suburban areas were randomly selected.

In total, 11727 out of 15648 invited individuals participated in the study. In total, 9720 participants completing dietary interviews were included in the final data analysis (Figure 2.4.).

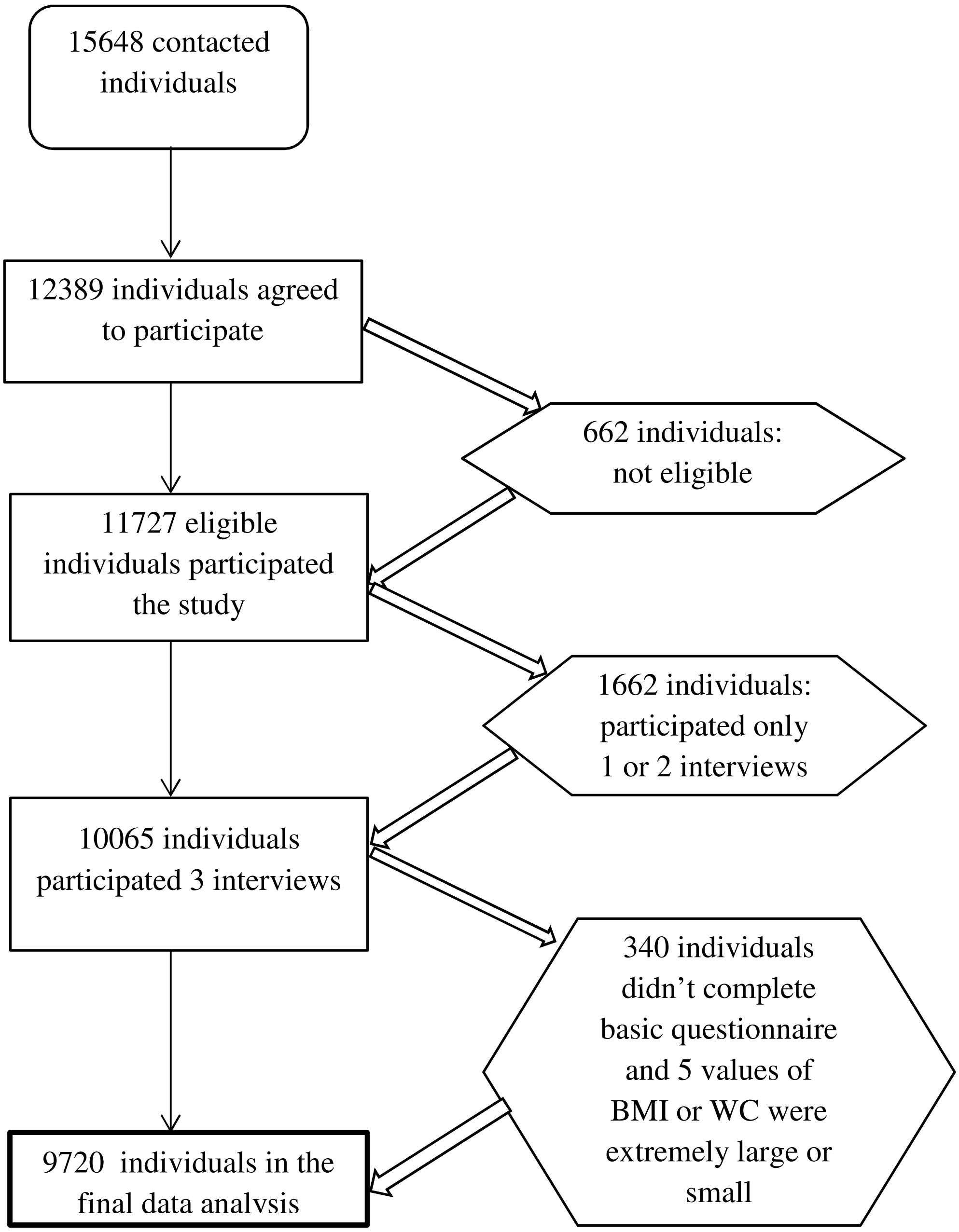

Figure 2.4. Participation and data selection of China study

- Data selection to the final data analysis

The basic criteria to select the target sample size into the final data analysis for all the studies were following:

- Subjects participated in all the 24-h dietary recall interviews (the Belgian population, European adolescents and the Chinese population) and estimated dietary records (Flemish preschoolers);
- Subjects reported basic demographic information such as age, gender, living regions;
- Dietary intakes of each subject were examined with the crosscheck during interviews. The cases of significance were re-contacted or excluded;
- Mean energy, protein and fiber intakes were checked between interviews by statistical analysis. The cases of significance were excluded;
- The cases were excluded if the self-reported body weight was significantly different from normal values or measured WC.

2. DIETARY ASSESSMENT

The 24-h dietary recall and EDR were the main dietary assessment methods used in this thesis to collect dietary information.

FPDS

3-d EDR were used to collect the detailed and quantitative dietary information, completed by the preschoolers' parents or proxies. Days were subdivided into six eating occasions, namely breakfast, morning snacks, lunch, afternoon snacks, dinner, and evening snacks. Detailed information on the type (including brand names) and portion size of the foods consumed was collected using an open entry format. In addition, parents were invited to give details on recipes, ingredients, and cooking methods.

The parents were not asked to weigh or measure the portion sizes of all foods consumed by their child. Instructions for correct completing the diet records were handed over to the parents [229]:

– Whenever the exact weight or volume was available in grams or ml, the portion sizes had to be recorded in these standard metrics;

– However, for some foods (like fruit), standard units/portions could be reported (e.g. 1 medium sized apple);

– For items or recipes that were difficult to quantify or to describe as standard units/portions (e.g. for drinks, spaghetti bolognaise, French fries, etc.), parents could use household measures like 'a small glass', 'half a plate'. Dieticians encoded the diaries, using a specific manual, based on the guide 'Maten en Gewichten, Handleiding voor een gestandaardiseerde kwantificering van voedingsmiddelen (Food portion sizes, manuel for standardized quantification)' and translated portion sizes into grams [120].

The staff supervising the children during lunchtime was asked to record the foods that the child had been eating during lunchtime. This information, together with the daily menu were given to the parents, who were asked to copy this information in the food diary of their child. Teachers coordinated to report to the parents whether the child had eaten/drunken the foods prepared by the parents and/or if they had been eating other food products during the breaks.

The distribution of 3-d EDR covered all days of the week and they were collected in autumn and winter. Only good quality EDR, containing adequate descriptions of the food products and portion sizes consumed, were included in the study. As a crosscheck, average energy and nutrient intakes were calculated as the mean of the three recorded days. Energy and some nutrients with significant estimated differences were rechecked by dieticians.

In total, 936 food items and composite dishes were encoded in the original database. All recipes that were described in depth as individual ingredients in the diaries were coded as ingredients. However, in order to classify foods easily into the food groups of the Flemish Food Based Dietary Guidelines (FBDG) [507], eight extra composite dishes had to be disaggregated (nasi goreng, nasi goreng with egg, spaghetti Bolognese, chicken ragout, turkey ragout, lasagna, macaroni ham/cheese sauce, and stew). Spaghetti Bolognese, for instance, was disaggregated into spaghetti, noodles, minced meat, onions, tomatoes, carrots, and margarine according to the recipe list of the Flemish European Prospective Investigation into Cancer and Nutrition software (EPIC-SOFT) version 2004 [124].

After the disaggregating procedures, food items were divided into 57 food groups, based on the classification of the Flemish FBDG and the expert opinion of the investigators. It should be noted that, due to lack of information, the complex food mixtures pizza (consumed by sixty-eight children during the three recorded days) and quiche (consumed by two children) were not disaggregated into their constituent components, but were categorized as a subcategory of the miscellaneous group.

BNFCS

Two non-consecutive 24-h dietary recall interviews were used to collect information on each participant's food consumption. The first 24-h dietary recall was obtained through a computer-assisted personal interview during a home visit by a trained dietitian. The second 24-h dietary recall was performed 2–8 weeks later during a second home visit (median 3 weeks). Interviews were randomly allocated to different days of the week and over a 12-month period in an effort to reduce within-person variation and to avoid seasonality effects. The 24-h dietary recalls collected information on the types and quantities of foods and beverages consumed over the preceding day to the interview. The dietitians used the European Prospective Investigation into Cancer and Nutrition software (EPIC-SOFT;

International Agency for Research on Cancer (IARC), Lyon, France) recently renamed GloboDiet to obtain standardized 24-h recall interviews. EPIC-SOFT was designed to obtain a detailed description and quantification of all foods and beverages consumed in a standardized way [451]. Quantification was facilitated using a picture book with colored photographs describing foods of different portion sizes [123]. The categorization of the food groups was defined based on the Data Food Networking (DAFNE) III project in order to make the results from the BNFCS can be comparable with other European Countries [483].

HELENA-CSS

Two non-consecutive computerized 24-h dietary recalls, instructed by dieticians/researchers, were used to collect food consumption data. During the self-administered interviews, adolescents were allowed to ask questions and after completion the recall was checked for completeness. Each participant was asked to fill in the HELENA- Dietary Assessments Tool (DIAT) twice in a time-span of 2 weeks.

HELENA-DIAT is a self-administered computer program based on the Young Adolescents' Nutrition Assessment on Computer (YANA-C) [504], consisting of a single computerized 24-h dietary recall with a structured program based on six meal occasions (breakfast, mid-morning snack, midday meal, afternoon snack, evening meal and evening snack). The validated YANA-C [504], was designed to obtain a detailed description and quantification of foods consumed, and eventually included more than 800 food items hierarchically organized in 25 food groups, and about 2600 colored photograph sets of more than 300 foods in different portions [503;505]. The food categorizations were based on the European Food Groups classification system [236].

CHNS

The three consecutive 24-h dietary recalls were used to collect individual dietary intake data regarding all food consumed away from home and at home, except for young children younger than 12 years of age, whose parents or proxies recalled their consumption. The start of the 3 consecutive 24-h recalls was randomly allocated from Monday to Sunday and almost equally balanced across the 7 days of the week from each sampling unit. Using food models and colored picture aids, well-trained interviewers recorded the types, amounts, type of meal, and place of consumption of all food items during the previous day [521]. The quality of the data collection was checked by comparing an individual's average daily dietary intake, calculated from the household survey and individual dietary intake based on 24-h recall data, respectively. Where significant discrepancies were found, the household and the individual in question were revisited and asked about their food consumption to resolve these discrepancies. Over 99% of the sample was available for the full 3d of data collection [572]. The food categorizations were based on Chinese dietary guidelines.

3. QUESTIONNAIRES

FPDS

General questionnaire

A general questionnaire was used to collect socio-demographic parameters and lifestyle characteristics for evaluating possible determinants of food consumption habits.

- Information on children including gender, physical activity level, special diet, supplements, consumption of lunch at school;
- Parental reported weight and height of the child;
- Information on parents including age, parental education levels, work status, birth countries, knowledge of national languages, physical activity, lifestyle;
- The family/household composition.

School questionnaire

The purpose of the school questionnaire was to evaluate of possible determinants for food consumption habits related to the schools or school policies. The school questionnaire, collecting additive information about the school policy and food supply, was completed by parents during a face-to-face interview with the director of the schools.

The main topics questioned in the school questionnaire are:

- Types of lunches (= meals taken during lunchtime) at school (e.g. possibility to have a hot meal or to bring their own bread meal …);
- Snacks/drinks (e.g. school milk) distributed at school;
- Vending machines (for snacks or drinks) at school;
- Nutritional education courses for preschool children at school;
- Nutrition campaigns recently held at school;
- Nutrition policy at school (e.g. whether snacks are allowed during the breaks, whether soft drinks are allowed …).

BNFCS

Participants completed questionnaire including demography, SES, heath status and lifestyle for the purpose to evaluate the nutritional adequacy of the food and nutrient consumption of Belgian population.

- Demographic section including gender, age, birth countries and cities, currently living place, the knowledge of national languages, marital status, family situation and family size;
- Heath status including physically and mentally health status, disease history, medication history;
- SES including education level, occupation, employment status;
- Lifestyle including smoking, consumption habits, sedentary lifestyle, physical activity;
- Others including pregnancy, self-reported weight and WC, self-reported weight and WC before pregnancy, the methods for losing weight and keep weight status.

HELENA-CSS

Adolescents completed self-reported questionnaires in the classroom. The questionnaires included general information, SES, nutritional knowledge test and food preference questionnaire to investigate their nutritional and health status.

General questionnaire: gender, age, birth country, knowledge of language, breast feeding, health status, physical activity level, knowledge of physicality activity, parental physical activity;

SES questionnaire: family size, family situation (e.g. house or apartment; own bedroom or shared bedroom; number of family cars) participant's and mother's nationality, language at home, body weight of parents, parental educational level, parental employment status, family's financial situation, the immigrant status of both participants and their parents;

Nutritional knowledge test questionnaire: energy intake and energy metabolism, physical activity, instrumental knowledge (e.g. nutrient contents) and knowledge of causal relationships (e.g. sweeteners or oral health).

CHNS

During an interview, adult participants needed to fill in a general questionnaire in order to evaluate the health and nutrition status of the Chinese population, and family planning policies.

Adult questionnaire

- Demography: gender, age, living province, living city, urban/rural, living years of the current place, the neighborhood situation, marital status, education level;
- Occupation: employment status (employed/unemployed/others), scale of the employer, type of occupation, years for the current job;
- Income status: source of income, income from occupation, monthly income, bonus of the last year;
- Lifestyle: smoking status, consumption of tea, coffee, alcohol, soft drink, physical activity;
- Family composition: parents, number of children
- Diet and activity knowledge: the knowledge of healthy diet and physical activity;
- Medical insurance: medical insurance status (yes/no), type of medical insurance;
- Health status and disease history;

Child questionnaire

Parents needed to report their child information about demography and lifestyle if the child was younger than 12 years old. Children, older than 12 years old, reported themselves the information.

- Demography: gender, age, living province, living city, urban/rural, living years of the current place, the neighborhood situation, relationship with parents, education level (primary school/ junior middle school/senior middle school/ technical school/ /university), and marital status of parents;
- Work status: full-time job, part-time job, looking for a job;
- Lifestyle: smoking status, consumption of tea, coffee, alcohol, soft drink, physical activity;
- Family composition: parents, number of siblings
- Diet and activity knowledge: the knowledge of healthy diet and physical activity;

- Medical insurance: medical insurance status (yes/no), type of medical insurance;
- Health status including first menstruation (for girl ≥ 8 years old) and disease history.

4. ANTHROPOMETRIC MEASUREMENTS

FPDS

Parents were asked to report the weight and height of their child in a questionnaire. Because of invalid parentally reported anthropometric data in the survey among Flemish preschoolers, which has been reported in details elsewhere [227], children's weight and height were not used to investigate the association with dietary protein and fiber intake in this thesis.

BNFCS

Weight (kg) and height (m) were self-reported by the participants during the interview. Pregnant women reported their weight before pregnancy. WC was measured in orthostatic position by the trained dietitians at home after 24-h recall interviews. Belts and thick clothing were removed, and the measuring tape was placed horizontally on the line crossing from the uppermost lateral border of the right iliac crest intersecting the axillary line.

HELENA-CSS

Adolescents' weight (kg) and height (m) were measured in underwear and barefoot during the interview. Weight was measured with an electronic scale (Type SECA 861) to the nearest 0.1 kg and height in the Frankfort plane with a telescopic height measuring instrument (Type SECA 225) to the nearest 0.1 cm by well-trained trained researchers.

A set of skinfold thicknesses (biceps, triceps, subscapular, suprailiac, thigh) and circumferences (relaxed arm, flexed upper arm, waist, hip, upper thigh) were measured three consecutive times on the left side of the body, with a Holtain caliper (to the nearest 0.2mm) and with a non-elastic tape (Seca 200) to the nearest 0.1 cm, respectively, according to Lohman's anthropometric standardization [312;345]. BF% was calculated using Slaughter's equations [448]. Physical maturations were examined by a physician during a medical examination to determine the pubertal status (stage I-V) based on Tanner and Whitehouse [473].

CHNS

Anthropometric measurements were carried out by trained health workers who followed standard protocol and techniques from WHO [296]. Body weight was measured to the nearest 0.01 kg in light indoor clothing to the nearest tenth of a kilogram with a beam balance scale. Height was measured to the nearest 0.10cm without shoes to the nearest tenth of a centimeter, using a portable stadiometer. WC was measured with an inelastic tape at a midpoint between the bottom of the rib cage and the top of the iliac crest at the end of exhalation [142].

Each of these measurements was carried out by at least two health workers, one worker took the measurements while a second health worker recorded the readings. Specific training on anthropometric measurement techniques was provided at the beginning of each survey, although most of the health workers have had previous experiences with other national health surveys.

5. BLOOD SAMPLING AND BLOOD ANALYSIS

HELENA-CSS

Blood samples were only collected in the HELENA-CSS in this thesis**.** Blood samples were collected in a subsample of the total sample participating in the HELENA-CSS. Adolescents involved in the blood sampling were asked to fast after 8 pm on the previous day. During blood sampling, 30 ml of fasting blood samples were drawn at school between 8:30 and 9:00 a.m. following a standardized blood collection protocol. In addition, a blood sampling questionnaire was completed by the participants for the purposes of assessing fasting status, acute infection, allergies, smoking, vitamin and mineral supplements, and medication, which was not used as criteria for exclusion of blood sample to the final data analysis.

Serum/plasma was centrifuged directly at the schools at 3500 rpm for 15 min at room temperature after blood collection. A specific handling transport and traceability system for biological samples were developed for the HELENA study. All samples were analyzed centrally. The serum samples collected at school were divided into two portions. One portion was transported without cooling within 24 hours to the central laboratory (IEL, Institut für Ernährungs- und Lebensmittelwissenschaften, Universität Bonn) and sent back to INRAN (Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Rome, Italy) on dry ice. The second portion was directly stored at -20 °C at INRAN on dry ice to the central laboratories and stored at -80°C until analysis.

Serum TC, triglycerides, LDL-C, HDL-C and glucose were measured on the Dimension RxL clinical chemistry system (Dade Behring, Schwalbach, Germany) with enzymatic methods using the manufacturer’s reagents and instructions. Serum CRP was measured using Enzyme-Linked ImmunoSorbent Assay (ELISA) according to the method described by Erhardt *et al* (2004) at INRAN (Rome) [153]. Serum insulin was analyzed at IEL (Bonn) by competitive immunoassay (Immulite 2000, DPC Biermann GmbH, Bad Nauheim, Germany). Serum leptin was measured using the RayBio Human Leptin ELISA (RayBiotech, Norcross, Georgia, USA) kit at UPM (Madrid).

CHAPTER 3

RESULTS FROM FLEMISH PRESCHOOLERS OF BELGIUM

CHAPTER 3.1

Dietary sources of animal and plant protein intake among Flemish preschool children and the association with socio-economic and lifestyle-related factors

Chapter based on this manuscript:

Lin Y, Bolca S, Vandevijvere S, Van Oyen H, Van Camp J, De Backer G, *et al*. Dietary sources of animal and plant protein intake among Flemish preschool children and the association with socio-economic and lifestyle-related factors. *Nutr J* 2011;10:97

1. ABSTRACT

Objectives

The aims of this study were to assess the intake of animal, plant and food group-specific protein, and to investigate their associations with socio-economic and lifestyle-related factors in Flemish preschoolers.

Methods

Three-day estimated dietary records were collected from 661 preschoolers aged 2.5-6.5 years (338 boys and 323 girls). Multiple linear regression analysis was used to investigate the association between animal, plant, and food group-specific protein intake and socio-economic and lifestyle factors.

Results

Animal proteins (mean 38 g/d) were the main source of total protein (mean 56 g/d), while mean plant protein intake amounted to 18 g/d. The group of meat, poultry, fish and eggs was the main contributor (51%) to animal protein intake, followed by milk and milk products (35%). Bread and cereals (41%) contributed most to the plant protein intake, followed by low-nutritious, energy-dense foods (21%). With higher educated fathers and mothers as reference, respectively, preschoolers with lower secondary and secondary paternal education had lower animal, dairy-, and meat-derived protein intakes, and those with lower secondary and secondary maternal education consumed less plant, and bread and cereal-derived proteins. Compared to children with high physical activity levels, preschoolers with low and moderate physical activity had lower animal and plant protein intakes. Significantly higher potatoes and grains-, and fish- derived proteins were reported for children of smoking mothers and fathers, respectively, compared to those of non-smoking mothers and fathers.

Conclusions

The total protein intake of Flemish preschoolers was sufficient according to the recommendations of the Belgian Superior Health Council. Parental level of education and smoking status might play a role in the sources of children's dietary proteins.

2. INTRODUCTION

Dietary protein is considered an important macronutrient, ideally contributing 10-15% to the total energy supply [551]. Protein is considered the most effective macronutrient in thermogenesis via the regulation of energy intake and satiety [74;197;253;313;528]. Moreover, proteins are vital for human metabolism as a source of essential amino acids [540].

High protein intakes have been associated to chronic diseases such as OB, MetS, hypertension, CVD, T2D, and kidney diseases [146;167;180;209;391;538]. Some studies showed that high consumption of total protein might help reducing body weight and improving bone health [146;167;180;538], whereas other studies reported a positive association between too high protein intake and children' s BMI-z-score [209].

Due to differences in *e.g.*, amino acids composition between animal and plant proteins, these two protein types act differently and should, therefore, be assessed separately [274;326]. A positive association was found between plant protein intake and health outcomes during childhood [7;14;209;274], whereas inverse associations were observed for animal protein intakes [194;418]. Gunther *et al.* (2007) found that higher animal protein intakes early in life, especially dairy protein intake, may be associated with an unfavorable body composition later in life, resulting in a higher risk for chronic diseases [194]. Recent results from the Dortmund Nutritional and Anthropometric Longitudinally Study suggested that a high animal protein intake during mid-childhood might be associated with an earlier pubertal growth spurt peak height velocity, while a higher plant protein intake, conversely, could delay puberty [193].

Parental involvement plays a critical role in promoting children's health behavior and dietary habits at an early age [394]. SES such as parental level of education and employment status, and more specifically household income might play a role in children's dietary preferences and choices of food quality [111;558]. Additionally, parental lifestyle might be an important factor in developing children's lifestyle and dietary habits. Recent studies indicated that parental smoking, maternal smoking in particular, is associated with a higher prevalence of OW and OB among children and early adolescents [421;511], yet without evidence whether children's dietary habits were influenced or not. Furthermore, studies on the relation between children's level of physical activity and pattern and food sources of protein intakes are currently missing.

Until now, only Guillaume *et al.* (2000) assessed the total protein intake among children aged 6-8 years living in the province of Luxemburg in Belgium [192]. However, no comprehensive data on the contribution from dietary animal and plant protein sources is available among Belgian preschoolers. Moreover, only a few studies report on the relation between dietary animal and plant protein intakes, and SES and lifestyle-related factors. The aims of the present study were, therefore, to evaluate dietary protein intake sources from the animal and plant-based foods in Flemish preschoolers, stratified in age-gender groups, and to identify their most important sources. Moreover, associations of total animal and plant, and food group specific protein intakes were examined with SES and lifestyle-related factors.

3. METHODS

3.1. Study population and design

The present study was obtained from the preschool dietary survey in the Flanders region of Belgium (October 2002 - February 2003), in which the usual dietary intake of preschoolers (2.5-6.5 years) was estimated from 3-d EDR completed by the parents. The participants were representative of Flemish preschoolers recruited from five provinces, including Antwerp, East-Flanders, West-Flanders, Flemish Brabant, and Limburg [229]. The exclusion criteria were: 1) staying in an institution (e.g., a hospital school) that provided the food, 2) not attending school during the whole period of the fieldwork, 3) living abroad (e.g. in the Netherlands) but attending school in Flanders, 4) having no Dutch speaking parent/proxy, and 5) having an older brother or sister participating in the study. The sampling design and methods have been described in detail previously [227;229], along with the response rate and the representativeness of the study sample (50% response rate and 49% after data-cleaning). In brief, a random cluster sampling design at the level of schools, stratified by province and age, was used. The proportion of the variance explained by schools and classes was low (<3%).

To ensure that all the days of the week would be covered approximately equally in the diet records, the research team determined beforehand the days randomly in order to avoid that the respondent would choose a day to record. Experienced dietitians performed the fieldwork during autumn and winter. The school headmasters, teachers and parents were informed about the study objectives and dietary assessment methods during a school meeting. Oral and written instructions were provided for the recording of foods and drinks consumed by children. Teachers were asked to report what the children consumed at school so that the parents/ proxies could include this information in the diaries.

The percentage of underreporters has been described in depth in a previous paper and was shown to be low (<2% of the children when using Goldberg cut-offs adapted for children) [228]. Underreporters were not excluded from the study sample for the present analyses because of low prevalence.

A total of 661 out of 1026 children (64%) who completed 3-d EDR, were included in the analysis for the present study. Among the 365 excluded children, 330 children did not complete the 3-d EDR correctly and 55 children's gender and/or age were missing.

The Ethical Committee of the Ghent University Hospital granted an ethical approval for the present study. Only children for whom a signed informed consent was obtained from (one of) the parents, were included in the study. More detailed information about the study design can be found elsewhere [227].

3.2. Socio-economic status and lifestyle-related factors

Parents were asked to fill out a questionnaire about the family background such as family size, family situation (two-parent family, one-parent family or special situation), level of parental education (lower secondary education, secondary education or higher education (bachelor, master or above) for mother and father), and employment (both parents employed, one parent employed or both parents unemployed), and lifestyle-related factors such as children's level of physical activity (low, moderate or high), and maternal and paternal smoking (yes/no).

Additionally, parents were asked to provide information on their children's age, gender, dietary habits, general health status, and whether the child had been breastfed or not. Also weight and height of the child were reported by the parents.

3.3. Dietary intake assessment

For the current analyses, only completed data from the 3-d non-consecutive EDR were used, excluding diaries containing insufficiently detailed descriptions of food products and/or portion sizes. Only diaries with three completed record days were included (n = 696; 66% of collected diaries). A record day was considered as incomplete if the portion size information was missing for most of the principal meal components (e.g. bread, beverages, etc.) or when the specifications about the food type (e.g. fat or skimmed milk) was missing for most of the principal meal components. Two dieticians with long-standing experience in nutritional epidemiological surveys, carried out this exclusion procedure. The distribution of 3-d EDR covered all days of the week and the autumn and winter season.

The food composition data for calculating the estimated protein intakes were derived from the following tables - in order of importance: the Belgian NUBEL [370], the Dutch NEVO [361], and the USDA [491] food composition databases, which used the Kjeldahl method for analyzing protein [30].

In total, 936 foods and composite dishes were encoded in the original database. All recipes that were described in depth as individual ingredients in the diaries were encoded as ingredients. However, in order to classify foods easily into the food groups according to the Flemish FBDG [507], eight additional composite dishes had to be disaggregated (fried rice, fried rice with eggs, spaghetti Bolognese, chicken ragout, turkey ragout, lasagna, macaroni with ham and cheese sauce, and stew). Spaghetti Bolognese, for instance, was disaggregated into spaghetti, minced meat, onions, tomatoes, carrots, and margarine, according to the recipe list of the Flemish EPIC-SOFT version 2004 [124]. However, since the ingredients of pizzas (consumed by sixty-eight children during the three recorded days) and quiches (consumed by two children) were seldom described in the diaries, we decided to categorize these food mixtures as a subcategory of pizza and quiches, instead of disaggregated into their constituent components. After the disaggregating procedures, food items were divided into 57 food subgroups of similar nutrient content or consumption, based on the classification of the Flemish FBDG and the expert opinion of the investigators

3.4. Statistical analysis

The descriptive analysis of energy and total, animal, and plant protein intakes, corrected for within-person variation, was performed by means of the Multiple Source Method (MSM) [127]. With this method, the total variance was adjusted for the intra-individual variances due to day-to- day variability.

Descriptive statistics of the study population are presented as the mean value or as the frequency distribution and standard deviation (SD) stratified by gender-age. Mean food group-specific energy-adjusted daily intakes were calculated based on the tertiles of total protein intake. The normality and equality of the variances were tested by the Kolmogorov-Smirnov test and Levene’s test, respectively. Comparisons of normally distributed data were performed with the Student’s t-test (between gender and age groups) or ANOVA (between tertiles of intake), whereas the non-parametric Kruskal-Wallis test was used to compare means of non-normally distributed data (between tertiles of intake).

To investigate the associations between total animal and plant, and food group-specific protein intakes, and SES and lifestyle-related factors, multiple linear regression analyses were performed (generalized linear regression (GLM)). Each model included SES (maternal and paternal level of education, parental employment, and family situation) and lifestyle-related factors (preschooler's level of physical activity, and maternal and parental smoking status) and was adjusted for potential confounding factors such as total energy intake, age, gender, nationality, and dietary supplement intake. Two-way interactions between potential confounding factors (total energy intake, age, gender) and independent variables (education of mother, education of father, parental employment, family situation, children's physical activity, smoking status of mother and smoking status of father), and between the potential confounding factors (total energy intake, age, gender) were created and examined. In the multiple linear regression analyses (GLM), the categories of higher educated mothers, higher educated fathers, unemployed parents, one-parent families, and non-smoking parents were considered as references. Significance of the associations was evaluated with the Type III Wald X^2 test. Outliers were removed based on residual plots. The statistical analysis was performed by SPSS software version 15.0 (Statistical Package for Social Sciences for Windows) and statistical significance for all the tests was set at a P value of 0.05.

4. RESULTS

4.1. Study population

Table 3.1.1 describes the socio-economic and lifestyle related factors, and nutrient intakes of the study population. The majority of the children were living with both parents. Approximately half of the parents had a higher education and about 70% of the children's parents were both employed. 30% of the preschoolers were living in Antwerp, 24% in East-Flanders, 22% in West-Flanders, 15% in Flemish Brabant, and 9% in Limburg (data not shown).

4.2. Total energy, total protein, and animal and plant protein intake

The energy derived from total proteins (mean: 224 kcal/d) contributed for 15.5% to the total energy intake (mean: 1455 kcal/d). Animal protein intake (mean: 38 g/d, range: 10.3-96 g/d) was the main contributor (69%) to total protein intake (mean: 56 g/d, 3.3 g/(kg*d), range: 26-125 g/d), while mean plant protein amounted to 17.5 g/d (range: 7.7-46 g/d). Energy, and total, animal, and plant protein intakes were higher in older preschoolers (4.0-6.5 years), especially in boys (Table 3.1.2). However, when considering the children's body weight, the younger (2.5-3.9 years) had significantly higher protein intakes than the older ($P < 0.001$). Girls at 4.0-6.5 years, consumed significantly less energy, and total protein, and animal and plant proteins than their male peers ($P < 0.001$, $P < 0.001$, $P = 0.013$, $P < 0.001$, respectively). Furthermore, in the age group of 2.5-3.9 years, the animal to plant protein ratio was significantly higher for girls than boys ($P = 0.025$).

Table 3.1.1. Socio-economic and lifestyle-related factors, and nutrient intakes of preschoolers in the Flanders preschool dietary survey

Characteristic	Total	Boys (n= 338)	Girls (n= 323)
		n (%)	
Age (n= 661)			
2.5-3.9 years	197 (29.8)	102 (30.2)	95 (29.4)
4.0-6.5 years	464 (70.2)	236 (69.8)	228 (70.6)
Socio-economic factors			
Family situation (n= 659)			
Two-parents family	632 (95.9)	323 (96.1)	309 (95.7)
One-parent family	23 (3.5)	11 (3.3)	12 (3.7)
Special situation	4 (0.6)	2 (0.6)	2 (0.6)
Maternal level of education (n= 655)			
Lower secondary	26 (4.0)	9 (2.7)	17 (5.3)
Secondary	250 (38.2)	127 (38.1)	123 (38.2)
Higher	379 (57.9)	197 (59.2)	182 (56.5)
Paternal level of education (n= 637)			
Lower secondary	49 (7.7)	25 (7.7)	24 (7.7)
Secondary	279 (43.8)	146 (45.1)	133 (42.5)
Higher	309 (48.5)	153 (47.2)	156 (49.8)
Parental employment (n= 634)			
Both parents employed	439 (69.2)	214 (66.0)	225 (72.6)
One parent employed	157 (24.8)	88 (27.2)	69 (22.3)
Both parents unemployed	38 (6.0)	22 (6.8)	16 (5.2)
Lifestyle			
Preschooler's level of physical activity (n= 652)			
Low	246 (37.7)	112 (33.4)	134 (42.3)
Moderate	303 (46.5)	163 (48.7)	140 (44.2)
High	103 (15.8)	60 (17.9)	43 (13.6)
Parental smoking status			
Smoking mother (n= 659)	98 (14.9)	54 (8.2)	44 (6.7)
Smoking father (n= 643)	160 (24.9)	82 (12.7)	78 (12.1)

Table 3.1.2. Mean total energy, and total protein, animal and plant protein intakes and their contribution to the energy intakes (n= 661)

Intake[a]	Total	Boys	Girls	P^b
Total energy intake (kcal/d)		Mean ± SD		
2.5-3.9 years	1408.4 ± 260.4	1441.7 ± 253.0	1372.7 ± 264.9	0.045
4.0-6.5 years	1474.4 ± 240.0*	1526.3 ± 233.7**	1420.8 ± 235.1	<0.001
Total protein (g/d)				
2.5-3.9 years	54.8 ± 11.8	55.2 ± 10.5	54.3 ± 13.0	0.405
4.0-6.5 years	56.4 ± 10.7	58.1 ± 10.3**	54.6 ± 10.9	<0.001
Total protein (g/(kg.d))				
2.5-3.9 years	3.7 ± 0.9	3.7 ± 0.8	3.7 ± 0.9	0.400
4.0-6.5 years	3.1 ± 0.8*	3.2 ± 0.7*	3.0 ± 0.8*	0.002
Animal protein (g/d)				
2.5-3.9 years	37.7 ± 10.9	37.2 ± 9.9	38.3 ± 11.8	0.576
4.0-6.5 years	38.8 ± 9.7	39.9 ± 9.6**	37.7 ± 9.8	0.013
Plant protein (g/d)				
2.5-3.9 years	17.0 ± 4.8	18.0 ± 5.6	15.9 ± 3.6	0.002
4.0-6.5 years	17.6 ± 4.4**	18.3 ± 4.5	17.0 ± 4.2**	0.001
Animal/plant protein ratio				
2.5-3.9 years	2.4 ± 0.8	2.2 ± 0.9	2.5 ± 0.9	0.025
4.0-6.5 years	2.3 ± 0.8	2.3 ± 0.7	2.3 ± 0.8	0.533
		Energy % ± SD		
Total protein				
2.5-3.9 years	15.7 ± 2.1	15.5 ± 2.1	15.9 ± 2.0	0.235
4.0-6.5 years	15.5 ± 2.1	15.4 ± 2.1	15.5 ± 2.0	0.642
Animal protein				
2.5-3.9 years	10.8 ± 2.4	10.4 ± 2.3	11.1 ± 2.4	0.043
4.0-6.5 years	10.6 ± 2.3	10.6 ± 2.3	10.7 ± 2.3	0.699
Plant protein				
2.5-3.9 years	4.9 ± 1.2	5.1 ± 1.4	4.7 ± 0.9	0.050
4.0-6.5 years	4.8 ± 0.9	4.8 ± 1.0	4.8 ± 0.8	0.971

SD, standard deviation.

[a]Mean total, animal, and plant protein intakes were adjusted for within-person variability using the MSM.

[b]*P* value for mean differences between boys and girls (Student's *t*-test after log transformation)

[*] Mean value was significantly different from 2.5-3.9 years old, $P \leq 0.001$ (Student's *t*-test after log transformation).

** Mean value was significantly different from 2.5-3.9 years old, $P < 0.05$ (Student's t-test after log transformation).

4.3. Food (sub)groups contributing to total, animal, and plant protein intake

The main contributor to the total protein intake among preschoolers was the group of meat, poultry, fish, and eggs, followed by milk and milk products, and bread and cereals (Table 3.1.3). The subgroups of meat, game and meat products, milk, flavored milk drinks, chicken and turkey, and cold cuts from meat products were the five most important contributors to animal protein intake. Bread and cereals contributed most to the plant protein intake, followed by low-nutritious, energy-dense foods, and potatoes and grains. Most of contribution to plant protein intake were derived from the subgroups of bread, rolls, crackers and rice cakes, sweet snacks, potatoes, cooked vegetables, and sugared bread. Noteworthy, the contribution of the group of soy products and soymilk to plant protein was three times higher in boys than girls.

The energy-adjusted daily intake of beverages, bread and cereals, and rest-group decreased based on the tertiles of total protein intake for both genders. The intake of dairy products, and meat, poultry, fish and eggs, however, increased significantly for both genders ($P < 0.001$; Table 3.1.4).

Table 3.13. Contribution from all food groups to total, animal, and plant protein intakes (n= 661)

Food group and subgroup[a]	Total protein				Animal protein				Plant protein			
	Total		Boys	Girls	Total		Boys	Girls	Total		Boys	Girls
	%	order[b]	%	%	%	order[b]	%	%	%	order[b]	%	%
Beverages (including juices)	**1.98**		**1.98**	**1.94**	**0.28**		**0.28**	**0.28**	**5.80**		**5.67**	**5.83**
Water	0.00		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00
Light beverages	0.03		0.04	0.03	0.00		0.00	0.00	0.10		0.12	0.09
Tea and coffee without sugar	0.00		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00
Tea and coffee with sugar	0.01		0.01	0.00	0.01		0.01	0.01	0.00		0.00	0.00
Fruit juice	1.20		1.21	1.23	0.00		0.00	0.00	3.90	6	3.81	4.06
Vegetable juice	0.00		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.01
Soup, bouillon	0.74		0.73	0.71	0.26		0.27	0.27	1.80		1.74	1.75
Soft drinks	0.00		0.00	0.00	0.01		0.00	0.00	0.00		0.00	0.01
Bread and cereals	**12.85**		**13.05**	**12.70**	**0.01**		**0.01**	**0.01**	**41.10**		**41.17**	**42.00**
Bread, rolls, crackers, rice cakes	10.34	4	10.54	10.20	0.00		0.00	0.00	33.00	1	33.26	33.74
Sugared bread	1.39		1.31	1.54	0.01		0.01	0.01	4.50	5	4.12	5.07
Breakfast cereals (ready-to-eat, hot)	1.12		1.20	0.96	0.00		0.00	0.00	3.60	8	3.79	3.19
Potatoes and grains	**3.94**		**3.86**	**3.98**	**0.15**		**0.13**	**0.15**	**12.40**		**11.91**	**12.81**
Pasta, noodles	0.94		0.85	1.03	0.00		0.00	0.00	3.00	10	2.68	3.41
Rice	0.36		0.35	0.36	0.00		0.00	0.00	1.20		1.11	1.18

Potatoes	2.64	10	2.66	2.59	0.15		0.13	0.15	8.20	3	8.12	8.22
Vegetables	**2.35**		**2.26**	**2.27**	**0.00**		**0.00**	**0.22**	**7.53**		**7.10**	**7.47**
Cooked vegetables	1.82		1.85	1.75	0.00		0.00	0.00	5.80	4	5.82	5.77
Raw vegetables	0.20		0.19	0.20	0.00		0.00	0.00	0.63		0.59	0.65
Vegetarian products (*e.g.* tofu, tempe,…)[c]	0.33		0.22	0.32	0.00		0.00	0.22	1.10		0.69	1.05
Fruits (sweetened and unsweetened)	**1.43**		**1.41**	**1.42**	**0.00**		**0.00**	**0.00**	**4.59**		**4.45**	**4.70**
Fresh fruit	1.21		1.18	1.21	0.00		0.00	0.00	3.90	6	3.73	4.01
Canned fruit	0.09		0.10	0.09	0.00		0.00	0.00	0.30		0.31	0.30
Dried fruit	0.02		0.01	0.02	0.00		0.00	0.00	0.05		0.04	0.06
Olives	0.00		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.01
Nuts and seeds	0.11		0.12	0.10	0.00		0.00	0.00	0.34		0.37	0.32
Soy products and soymilk	**1.13**		**1.70**	**0.53**	**0.02**		**0.01**	**0.03**	**3.63**		**5.38**	**1.66**
Soy drinks	0.99		1.53	0.44	0.00		0.00	0.00	3.20	9	4.84	1.44
Soy-based desserts	0.13		0.17	0.07	0.00		0.00	0.00	0.43		0.54	0.22
Fermented milk or soy drinks (*e.g.* actimel, yakult,…)	0.01		0.00	0.02	0.02		0.01	0.03	0.00		0.00	0.00
Milk and milk products	**24.53**		**24.24**	**24.84**	**35.14**		**34.94**	**35.00**	**1.87**		**1.75**	**1.91**
Milk (including goat's milk)	11.31	2	11.20	11.44	16.60	2	16.57	16.53	0.00		0.00	0.00
Flavoured milk drinks (*e.g.* fristi, chocolate milk,…)	10.48	3	10.20	10.68	14.60	3	14.36	14.67	1.70		1.57	1.74
Yoghurt	0.35		0.34	0.36	0.52		0.50	0.52	0.00		0.00	0.00

Sugared or aromatised yoghurt	1.01		1.12	0.94	1.50		1.62	1.34	0.07	0.07	0.06
Milk desserts	1.37		1.37	1.40	1.90	9	1.87	1.92	0.10	0.09	0.11
Cream	0.01		0.01	0.02	0.02		0.02	0.02	0.00	0.02	0.00
Cheese	**7.46**		**7.11**	**7.78**	**10.87**		**10.44**	**11.15**	**0.11**	**0.11**	**0.11**
Hard cheese (no cream cheese)	5.16	7	4.71	5.50	7.60	6	6.97	7.95	0.00	0.00	0.00
Fresh cheese	1.61		1.70	1.56	2.30	8	2.46	2.21	0.10	0.11	0.10
Cheese spread	0.69		0.70	0.72	0.97		1.01	0.99	0.01	0.00	0.01
Fat and oil	**0.08**		**0.08**	**0.07**	**0.03**		**0.03**	**0.02**	**0.20**	**0.20**	**0.20**
Butter, margarine	0.08		0.08	0.07	0.03		0.03	0.02	0.20	0.20	0.20
Oil	0.00		0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00
Frying oil	0.00		0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00
Meat, poultry, fish, and eggs	**34.66**		**34.78**	**34.84**	**50.58**		**50.74**	**49.95**	**0.39**	**0.35**	**0.43**
Meat, game, meat products	15.58	1	15.75	15.56	23.12	1	23.00	22.34	0.06	0.05	0.08
Chicken, turkey	8.01	5	8.28	7.85	11.50	4	11.97	11.24	0.01	0.01	0.00
Fish, shellfish	2.99	9	3.01	2.87	4.20	7	4.33	4.01	0.30	0.27	0.33
Cold cuts from meat products	6.52	6	6.27	6.84	9.50	5	9.27	9.88	0.02	0.02	0.02
Cold cuts from fish products	0.31		0.27	0.31	0.46		0.40	0.44	0.00	0.00	0.00
Eggs[d]	1.26		1.20	1.41	1.80	10	1.77	2.04	0.00	0.00	0.00
Restgroup (snacks and desserts)	**8.93**		**8.89**	**8.88**	**3.01**		**2.94**	**3.07**	**21.43**	**20.95**	**21.81**
Brioches	0.43		0.46	0.36	0.01		0.01	0.02	1.37	1.43	1.15

Sweet snacks (*e.g.* waffle, apple pie)	4.97	8	5.02	4.90	1.30		1.30	1.34	12.50	2	12.28	12.74
Salty snacks (*e.g*, cheese biscuits)	0.24		0.20	0.27	0.04		0.03	0.04	0.67		0.57	0.78
Salty sauces	0.56		0.52	0.58	0.48		0.44	0.52	0.71		0.69	0.71
Sweet sauces	0.00		0.00	0.01	0.00		0.00	0.00	0.01		0.00	0.02
Chocolate	0.43		0.38	0.47	0.23		0.20	0.25	0.84		0.74	0.96
Chocolate spread	0.96		0.95	0.95	0.51		0.50	0.51	1.97		1.94	1.98
Other sweet spread (*e.g.* jam, honey, …)	0.05		0.04	0.05	0.00		0.00	0.00	0.15		0.14	0.18
Sugar	0.00		0.00	0.00	0.00		0.00	0.00	0.00		0.00	0.00
Fried snacks	0.01		0.01	0.02	0.00		0.00	0.00	0.00		0.00	0.00
French fries, croquettes	0.93		0.92	0.96	0.01		0.00	0.02	3.00		2.91	3.12
Sweet desserts (*e.g.* ice cream, tiramisu, …)	0.35		0.39	0.31	0.43		0.46	0.37	0.21		0.25	0.17
Miscellaneous	**0.64**		**0.60**	**0.71**	**0.36**		**0.30**	**0.40**	**0.96**		**0.94**	**1.02**
Pizza and quiches	0.38		0.38	0.36	0.31		0.30	0.30	0.52		0.55	0.51
Others[e]	0.26		0.22	0.35	0.05		0.00	0.10	0.44		0.39	0.51

[a]These mean food group and subgroup-specific intakes are rough estimates calculated from the raw data on which these nutrient contributions are based. The high number of non-consumers in some of the food (sub)groups hindered the adjustment for within-person variation.

[b]Ranking of the 10 food subgroups with the highest contribution to the total, animal or plant protein intakes.

[c]Includes tofu, quorn and pulses.

[d]Includes eggs reported separately and eggs included in disaggregated food mixtures.

[e]Includes foods or components with negligible contributions to the total nutrient intakes that could not be categorized in the above food (sub)groups (e.g. quiches herbs and spices, monosodium glutamate, starch, plain gelatin, artificial sweeteners, pectin and cocoa powder).

Table 3.1.4. Mean energy-adjusted daily intakes (g/d) of different food groups based on tertiles† of total protein intake (n= 661)

	Total			Boys			Girls			P^a		
Food groups	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**	**Total**	**Boys**	**Girls**
Beverages	279.0	243.9	225.4	271.0	249.9	227.9	284.4	236.6	221.8	0.001	0.116	0.008
Bread and cereals	61.5	61.0	56.4	62.7	62.7	57.9	60.7	58.8	54.2	0.028	0.165	0.087
Potatoes and grains	59.7	60.3	60.0	62.4	58.7	59.3	57.9	62.3	60.9	0.976	0.637	0.529
Vegetables	46.2	45.6	51.2	45.2	42.8	53.6	46.9	48.9	47.7	0.256	0.028	0.435
Fruits	69.2	72.3	82.0	72.6	65.3	79.7	66.9	80.7	85.3	0.037	0.095	0.073
Soy products and soymilk	9.6	13.9	15.0	12.3	18.3	25.0	7.8	8.6	1.1	0.763	0.516	0.253
Dairy products[b]	239.2	295.4	362.2	223.4	285.9	351.2	250.0	306.8	377.5	<0.001	<0.001	<0.001
Meat, poultry, fish and eggs	52.7	60.0	69.7	52.8	58.6	67.9	52.7	61.8	72.3	<0.001	<0.001	<0.001
Restgroup	117.9	98.5	92.4	133.0	105.3	102.0	107.6	90.2	79.0	0.014	0.033	0.045

†Tertiles based on total protein intake (g/d) among Flemish preschoolers. Tertile 1 (T1): total protein intake <51 g/d; tertile 2 (T2): 52 g/d < total protein intake <60 g/d; tertile 3 (T3): total protein intake ≥60 g/d.

[a]*P* value for mean differences between T1, T2, and T3 (ANOVA for the food groups beverages, bread and cereals, potatoes and grains, dairy products, and meat, poultry, fish and eggs; Kruskal-Wallis test for the food groups vegetables, fruits, soy products and soymilk, and restgroup).

[b]Dairy products include the food groups milk and milk products, and cheese.

4.4. Associations between protein intakes and socio-economic status and lifestyle-related factors

Associations between animal, plant, and food group specific protein intakes, and socio-economic and lifestyle related factors were examined after adjustment for potential confounding factors including energy intake, gender, age, nationality, and dietary supplement use, and with higher educated parents, preschoolers with a high level of physical activity, families with both parents unemployed, one-parent families, and non-smoking parents as references (Table 3.1.5). Inverse associations were found between animal protein intake and the paternal level of education, and the preschoolers' level of physical activity and between plant protein intake and the maternal level of education, and the children's level of physical activity. Additionally, protein intakes derived from dairy and meat sources were inversely associated with the paternal level of education, whereas, compared to children of higher educated mothers, preschoolers of lower secondary or secondary educated mothers had higher animal protein intakes through the consumption of poultry and fish. Moreover, fish-derived protein intakes were also positively associated with smoking fathers. Bread and cereal-, and vegetable derived protein intakes were inversely associated with the maternal level of education; whereas potato and grain derived protein intakes were positively associated with smoking fathers. Vegetable- and fruit-derived protein intakes were also inversely associated with the paternal level of education. Children with one employed parent consumed more vegetable proteins compared to those with both parents being unemployed. Furthermore, the inclusion of interaction terms in the model showed significant interactions, between maternal/paternal level of education * total energy intake and between smoking mother*total energy intake. These interactions show that total energy intake and SES and lifestyle factors affect each other's impact on the outcome variables (animal, plant protein and protein sources (meat, poultry, bread and cereal, and potato and grain)) (data not shown).

Table 3.1.5. Associations between protein intakes and socio-economic and lifestyle-related factors by Generalized linear model

									<u>**Animal protein and specific main sources**</u>											
Predictor variables[a,]	**Total animal protein**				**Dairy protein[b]**				**Meat protein[c]**				**Poultry protein**				**Fish protein**			
	B	**95 % CI**		**P**	B	**95 % CI**		**P**	B	**95 % CI**		**P**	B	**95 % CI**		**P**	**β**	**95 % CI**		**P**
Parental education																				
Lower secondary educated mother	15.68	-12.20	43.55	0.270	-3.00	-6.39	0.39	0.083	-6.33	-15.37	2.71	0.170	0.30	-9.79	10.40	0.953	1.11	0.16	2.05	0.022
Secondary educated mother	6.42	-4.12	16.96	0.233	-0.13	-1.56	1.30	0.859	0.22	-3.64	4.08	0.910	3.96	0.04	7.89	0.048	0.12	-0.25	0.49	0.534
Lower secondary educated father	-1.22	-4.05	1.62	0.400	-4.14	-6.53	-1.74	0.001	-8.65	-15.58	-1.71	0.015	-3.26	-15.76	9.24	0.609	-1.71	-7.62	4.20	0.570
Secondary educated father	-1.77	-3.40	-0.15	0.032	-1.94	-3.22	-0.66	0.003	-3.77	-7.17	-0.37	0.030	-4.47	-10.83	1.89	0.168	-0.55	-3.55	2.46	0.722
Parental employment																				
Both employed parents	2.88	-27.05	32.80	0.850	-0.57	-3.49	2.35	0.701	11.27	-1.42	23.97	0.082	0.54	-0.82	1.90	0.438	5.64	-1.47	12.76	0.120
One employed parent	1.33	-30.31	32.96	0.934	0.91	-2.21	4.03	0.567	11.47	-1.95	24.89	0.094	0.07	-1.38	1.52	0.922	7.19	-0.33	14.72	0.061
Family situation																				
Two- parent family	4.57	-56.85	65.99	0.884	4.17	-2.04	10.37	0.188	-0.98	-27.04	25.07	0.941	5.95	-24.94	36.84	0.706	5.62	-8.98	20.23	0.451
Lifestyle																				
Light physical activity	-4.42	-7.51	-1.33	0.005	-1.20	-3.04	0.65	0.204	0.06	-0.77	0.89	0.892	2.30	-6.24	10.83	0.598	-1.37	-6.53	3.79	0.602
Moderate physical activity	-3.39	-6.47	-0.31	0.031	-0.94	-2.72	0.85	0.305	-0.08	-0.88	0.72	0.839	-4.07	-12.25	4.11	0.330	-4.41	-9.41	0.60	0.084
Smoking mother	7.44	-10.50	25.38	0.416	-0.83	-2.81	1.16	0.414	1.03	-6.58	8.64	0.791	-2.59	-11.61	6.43	0.574	1.93	-2.34	6.20	0.375
Smoking father	7.49	-5.02	20.00	0.241	-0.22	-1.83	1.39	0.788	-0.47	-5.78	4.84	0.862	-2.56	-8.85	3.73	0.426	0.67	0.11	1.23	0.019
									<u>**Plant protein and specific main sources**</u>											
	Total plant protein				**Cereal protein**				**Potato and grain protein**				**Vegetable Protein**				**Fruit Protein**			
Parental education																				
Lower secondary educated mother	-14.29	-23.43	-5.15	0.002	-9.67	-15.19	-4.15	0.001	0.58	-2.86	4.03	0.741	-0.12	-0.40	0.17	0.421	-0.25	-0.65	0.15	0.223
Secondary educated mother	-4.08	-7.63	-0.53	0.024	-0.96	-3.07	1.15	0.373	-0.34	-1.79	1.11	0.647	-0.15	-0.26	-0.03	0.012	-0.03	-0.19	0.14	0.750
Lower secondary educated father	-2.62	-13.97	8.73	0.652	0.47	-0.31	1.26	0.239	-2.30	-5.04	0.44	0.099	-0.14	-0.34	0.06	0.159	-0.16	-0.43	0.11	0.248
Secondary educated father	3.41	-2.36	9.19	0.247	0.21	-0.27	0.69	0.397	0.31	-1.08	1.70	0.663	-0.14	-0.25	-0.02	0.020	-0.22	-0.36	-0.07	0.004
Parental employment																				
Both employed parents	-0.02	-1.45	1.40	0.975	-2.18	-10.31	5.96	0.600	-1.76	-5.06	1.54	0.295	0.05	-0.17	0.27	0.657	-0.01	-0.34	0.31	0.928
One employed parent	0.26	-1.26	1.78	0.738	0.15	-8.45	8.75	0.973	-1.97	-5.45	1.52	0.269	0.25	0.02	0.49	0.034	0.02	-0.32	0.37	0.900

Family situation																				
Two- parent family	-0.91	-2.52	0.69	0.265	-2.12	-18.81	14.57	0.804	-3.11	-9.88	3.65	0.367	-0.16	-0.63	0.30	0.490	-0.01	-0.69	0.67	0.981
Lifestyle																				
Light physical activity	-1.61	-6.17	2.94	0.488	0.96	-2.00	3.92	0.524	-0.10	-0.32	0.11	0.347	-0.07	-0.21	0.08	0.373	-0.09	-0.30	0.12	0.399
Moderate physical activity	-4.93	-9.40	-0.45	0.031	-1.05	-3.94	1.83	0.474	-0.14	-0.35	0.08	0.212	0.03	-0.11	0.17	0.672	0.02	-0.18	0.22	0.830
Smoking mother	0.77	-7.42	8.96	0.854	-0.20	-5.08	4.67	0.936	1.34	0.11	2.63	0.033	0.09	-0.06	0.24	0.232	-0.03	-0.24	0.19	0.811
Smoking father	-1.58	-7.29	4.14	0.588	-0.38	-0.84	0.07	0.098	0.40	-0.98	1.78	0.568	0.03	-0.10	0.15	0.690	-0.08	-0.25	0.09	0.353

B: coefficient B; CI, confidence interval; NS, not statistically significant.

[a]GLM was controlled for total energy intake, age, gender, nationality, and dietary supplement intake. Higher educated parents, families with both parents unemployed, preschoolers with a high level of physical activity, and non-smoking parents were used as references.

[b]Dairy products include the food groups milk and milk products, and cheese.

[c]Meat refers to the food subgroup meat, game, and meat products.

5. DISCUSSION

To the best of our knowledge, this is the first study that assessed dietary animal and plant protein intake from different food sources in Flemish preschoolers. Moreover, this is the first study to investigate associations between animal, plant, and food group-specific protein intakes, and SES and lifestyle-related factors. The current study shows that the most important contributor to total protein intake among Flemish preschoolers was meat, followed by dairy products, and bread and cereals.

5.1. Total energy, and animal, plant, and food group-specific protein intake

Our results show that all but one boy (4.0-6.5 years) met the recommended dietary allowances (RDA) for protein intake set by the BSHC (2.5-3.9 years: 0.86-0.97 g/(kg*d), 4.0-6.5 years: 0.85-0.91 g/(kg*d)) [160], and the WHO/FAO/UNU (10.0-15.0% of total energy) [551]. Moreover, 58% preschoolers (2.5-3.9 years: 118 children; 4.0-6.5 years: 265 children) exceeded the RDA for total protein intake.

A previous Belgian study, also using 3-d EDR among 6-8 years old children living in the province of Luxembourg, reported considerably higher dietary energy and total protein intakes (boy: 2308 kcal/d, girls: 2254 kcal/d; 67 g/d for both genders) than our study results (boys: 1501 kcal/d, girls: 1407 kcal/d; boys: 57 g/d, girls: 55 g/d) [192]. Due to the lack of information on total, animal, and plant protein intakes in Belgian preschoolers, we relate our findings to those available on other countries including UK (4d weighted-food records), Germany (3-d EDR), Spain (two 24-h recalls), and the US (combination of 24h recall and 2d food records) [32;194;431;446]. Compared to our study population, lower energy intakes were reported for German children aged 1.5-6 years (928-1398 kcal/d) [194] and British children aged 1.5-4.5 years (boys: 1175 kcal/d; girls: 1098 kcal/d) [32], but in Spain (1595 kcal/d) [431] and the US (boys: 1458-1728 kcal/d; girls: 1356-1576 kcal/d) [446], children aged 2-5 years had higher intakes. When comparing the total protein intakes between these populations, a pattern similar to that of the energy intake was observed for the total protein intakes as such, but not for the energy contribution from total protein. On the one hand, total protein intakes observed in the present study were comparable to those among children in the US (boys: 50-61 g/d; girls: 49-51 g/ d) [446], but higher than those reported for British (boys: 36 g/d; girls: 33 g/d) [32] and lower than those of Spanish children (66 g/d) [431]. On the other hand, the energy percentage from total protein contributed more in our study population than for German (12.4-13.8%) [194] and British (boys: 12.1%; girls: 12.0%) [32] children. Furthermore, the relative energy contributions from animal and plant proteins were also lower in German children (7.8-9.3%, 4.2-4.5%, respectively) [194].

Concerning the food sources contributing to total protein intake, energy percentage of protein-derived from meat, dairy (including cheese), and bread and cereals (including bread, breakfast cereals, pasta, rice, and flour) were higher in the present population (3.5%, 4.8%, 2.7%, respectively) (data not shown) compared to the German preschoolers (2.5-2.7%, 3.5-4.1%, and 2.4-2.7%, respectively) [194]. Milk and flavoured milk drinks-,meat-, poultry-, pasta-, and French fries and chips derived protein intakes of Flemish preschoolers were considerably lower than those of American children (25%, 17.4%, 10.3%, 2.7%, and 0.8%, respectively) [446], but substantially higher than those of Spanish children (10.2%, 10.7%, 4.5%, and 4.2%, respectively) [415]. On the other hand, the contributions from yoghurt, and fish and shellfish to the total protein intake were significantly lower in Belgium than in Spain (6.9% and 4.3%, respectively), but much higher than in the US (1.1% and 1.5%, respectively). Additionally, eggs, breakfast cereals, pasta, and nuts and seeds contributed much more to the total protein intake in the US (2.8%, 3.1%, 2.7%, and 2.5%, respectively) than in Belgium, while cold cuts from meat products contributed much less in the US (1.0%). Conversely, bread and cheese contributed considerably more to the total protein intake in Belgium than in the US (10.0% and 5.8%, respectively) and Spain (6.5% and 4.3%, respectively). Noteworthy, legumes contributed substantially to the total protein intake of Spanish children (3.9%), but it had similar contribution to Belgian (1.1%) and US children (1.0%).

To summarize, we observed that the food sources contributing to protein intake in Flemish preschoolers were mainly from animal origin such as meat, game, meat products, milk and cheese, and low-nutritious, energy-dense food (sweet snack in particular), whereas plant sources, including vegetables, fruit, and breakfast cereals, had much lower contributions. Therefore, according to the literature, Flemish preschoolers do not have very healthy dietary habits compared to other countries, as they consume more unhealthy foods such as French fries and chips, cold cuts from meat products, and less vegetables, fruit, legumes, cereals, and fish. Hence, the food sources contributing to the total protein intake among Flemish preschoolers are narrow and limited.

5.2. Associations between animal, plant, and food group-specific protein intakes and socio-economic status and lifestyle-related factors

To the best of our knowledge, there is no data available on the associations between SES and lifestyle-related factors, and dietary protein intakes derived from animal and plant based foods in general or from particular food groups among Flemish preschoolers.

In this study, with higher educated fathers and mothers as reference respectively, children with (lower) secondary educated fathers had lower animal, dairy-, meat-, vegetable-,and fruit-derived protein intakes, whereas children with (lower) secondary educated mothers consumed less plant, bread and cereal-, and vegetable-derived proteins and more poultry- and fish-derived proteins. Additionally, paternal and maternal smoking was positively associated with fish-, and potato and grain-derived protein intakes, respectively. These findings are in line with previous studies indicating that parental SES and lifestyle-related factors are directly associated with children's dietary behavior [111;211;558;559]. Recent studies show that children from parents with a higher SES, are more likely to have healthier protein patterns (more cereals, fruit and vegetables), whereas more unhealthy protein patterns, defined by a high proportion of animal proteins, are found in families with a lower SES [111;117;152;226]. Furthermore, the choice of good-quality protein sources may be a critical factor as well due to differences in amino acid content. Apparently, due to the higher cost, families with a high SES purchase more high-quality food such as lean meat, fish, vegetables and fruits than families with a low SES [558]. In our study, children with one employed parent consumed more vegetables proteins than those with both parents being unemployed.

Furthermore, we found that children having a low or moderate level of physical activity had lower animal and plant protein intakes than those having high levels of physical activity. Moreira *et al.* (2010) reported that sport activities were positively associated with the dietary intake of fish, meat, eggs, vegetables, bread, yoghurt, and cheese [339]. Children's level of physical activity, however, might be influenced by parental education [24;339]. Aranceta *et al.* (2003) found, after controlling for parental education, that children of less educated mothers spending more than 2 h/d on television watching were more likely to follow the 'Snack' pattern [339]. Therefore, parental level of education, maternal in particular, plays an important role in the development of children's eating behavior and lifestyle [24]. Yannakoulia *et al.*

(2008) suggested that children in two-parent families have more chances to have regular meals and healthy foods than children from divorced parents [567].

5.3. Strengths and limitations

The present study was the first investigating dietary animal and plant protein intakes in Flemish preschoolers. The results of this large cross-sectional study represent the Flemish preschoolers' animal and plant protein intakes with a good accuracy and validity because of the low prevalence of underreporters (2%) and the high coverage of all five Flemish provinces. Like all studies, some limitations should also be taken in to consideration.

First, the method of 3-d EDR represents only the individual children's short-term daily intake rather than usual intake. However, we corrected for within-person variability by using the MSM method to get more precise usual daily protein intakes. Under- or overestimation might influence the true portion sizes, which makes the estimated animal and plant protein intakes less accurate.

In addition, it should be noted that the food composition data, used for calculating dietary protein intake, might as well have introduced some bias in the estimated nutrient contributions [109].

Although all days of the week were covered in our dietary survey, it was impossible to correct for seasonal variations completely, because our fieldwork was conducted only during autumn and winter. However, low seasonal variations influencing dietary intakes in the Belgian population was reported because the widespread availability of most foods all year round [124].

Furthermore, data of SES and lifestyle-related factors were reported by the preschoolers' parents. Therefore, we had to rely on the parents' memory and ability to estimate some lifestyle-related factors such as the frequency and duration of preschoolers' physical activity. Additionally, parents needed to estimate the level of physical activity based on their own definition. Selection bias might lead to bias of imprecise associations [229]. For example, lower educated parents might be unwilling to report their highest level of education.

6. CONCLUSIONS AND RECOMMENDATIONS

To conclude, the total dietary protein intake of almost all preschoolers met the RDA of the BSHC. Meat was the most important contributor to total protein intake, followed by dairy, and bread and cereals. Furthermore, the results show that animal and plant protein intakes were inversely associated with the paternal and maternal level of education, respectively, and children's level of PA, and that some food-group specific protein intakes were associated with the parental level of education, smoking and/or employment status. SES and lifestyle-related factors, parental education in particular, seem, therefore, to play a role in the development of children's dietary animal and plant protein intakes.

Although the total protein intakes reached the RDA of the BSHC and WHO, we noticed that the food sources in our study population were narrow and mainly from animal origin rather than from plants. Hence, parental involvement could help to establish healthy food choices in preschoolers. It is important to inform and educate lower educated parents about healthy food habits for their children since an early health-related knowledge and lifestyle can be adopted under parental influence [185]. However, pressuring children to eat and restricting access to specific foods is not recommended because it often leads to overeating, dislikes, and interest in forbidden items [185].

CHAPTER 3.2

Dietary sources of fiber intake and its association with socio-economic factors among Flemish preschool children

Chapter based on this manuscript:

Lin Y, Bolca S, Vandevijvere S, De Keyzer W, Van Oyen H, Van Camp J, *et al*. Dietary Sources of Fiber Intake and Its Association with Socio-Economic Factors among Flemish Preschool Children.

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1. ABSTRACT

Objectives

To assess total dietary fiber intake, identify the major sources of dietary fiber, and examine its association with socio-economic factors among Flemish preschoolers.

Methods

Three-day estimated dietary records were collected from a representative sample of preschoolers 2.5-6.5 years old (n= 661; 338 boys, 323 girls).

Results

The mean dietary fiber intake (13.4 g/d) was lower than the intake level recommended by the Belgian Superior Health Council (70% boys and 81% girls below the guidelines). The most important contributor was the group of bread and cereals (29.5%), followed by fruits (17.8%), potatoes and grains (16.0%), energy-dense, low-nutritious foods (12.4%), and vegetables (11.8%). Multiple linear regression analyses showed that total fiber intake was associated with maternal education and parents' employment. Overall, fiber intakes from high-nutritious foods (vegetables and fruits) were higher in preschoolers of higher educated mothers and those with one or both parents being employed.

Conclusion

The majority of the preschoolers had dietary fiber intakes below the recommended level. Hence, dietary fiber should be promoted among parents of preschoolers and low SES families should be addressed in particular.

2. INTRODUCTION

A significantly decreased DF intake and concomitant increased intake of total fat, saturated fatty acids and cholesterol in industrialized countries was found to be associated with a higher prevalence of chronic diseases [94;354]. WHO identified a low DF intake as an important determinant for chronic diseases, including OB, CVD, and diabetes [551]. DF is one of the nutritional compounds of vegetables, fruits, legumes, nuts, and whole-grain foods, known as carbohydrate polymers with ten or more monomeric units, that are not hydrolyzed by endogenous enzymes in the small intestine [115].

Evidence shows that a higher intake of DF is significantly associated with lower BMI, systolic and diastolic blood pressure, serum LDL-C and TG [4;84;118;140;201]. Hence, a sufficient intake of DF is strongly recommended by the BSHC [160], WHO [551], USDA [489], and British Nutrition Foundation [75]. Many chronic diseases and some cancers in adults have been related to dietary factors during early childhood [168;416]. Williams & Bollella (1995) reported that a higher DF intake may have a positive effect on serum vitamin and mineral concentrations in healthy children consuming a balanced diet containing adequate levels of nutrients [534].

In addition, as dietary habits are established in early life, young children need to be encouraged to consume nutritious, fiber-rich foods daily to achieve an optimum health status [534]. DF intake of European preschoolers is poorly documented. Reported DF intakes in children and adolescents range from 0.9 to 3.5 g /MJ, although, different analytical methods or definitions were used [284]. A recent small-scale Flemish study (115 children, 2-3 years) [71] reported that the DF intake of children did not reach the recommendations of the BSHC [160]. As far as we are aware, no previous study has undertaken a comprehensive analysis of the food sources of DF among Belgian preschoolers. Furthermore, children and adolescents from low SES families were previously found consuming less DF, but higher energy-dense foods with higher risk of OW and OB [7;226;251;287;536]. Due to lack of knowledge on DF consumption in Belgian preschoolers, the present study aimed to assess the DF intake, and to identify the major food sources of DF among Flemish preschoolers. Furthermore, the association between total and food group-specific fiber intakes and SES was examined.

3. METHODS

3.1. Survey population

This study used data from the Flanders preschool dietary survey (data collected from October 2002 until February 2003), in which the usual dietary intake of Flemish preschoolers (2.5-6.5 years) was estimated from 3-d EDR, completed by the parents. The distribution of 3-d EDR covered the whole week in autumn and winter. The sampling design and methods have been described in detail previously, along with the response rate and representativeness of the study sample (50% response rate and 49% after data-cleaning) [229]. In brief, a random cluster sampling design at the level of schools, stratified by province and age, was used [229].

To ensure that all the days of the week would be covered approximately equally in the diet records, the research team determined beforehand the days randomly in order to avoid that the respondent would choose a day to record. Experienced dietitians performed the fieldwork during autumn and winter. The school headmasters, teachers and parents were informed about all the study objectives and dietary assessment methods during a school meeting. Oral and written instructions were provided for the recording of foods and drinks consumed by children. Teachers were asked to report what the children consumed at school so that the parents/proxies could include this information in the diaries.

The percentage of underreporters has been described in depth in a previous paper and was shown to be low (< 2% when using Goldberg cut-offs adapted for children) [228]. Underreporters were excluded from the study sample for the analyses described in this paper.

The Ethical Committee of the Ghent University Hospital (Belgium) granted ethical approval for the study. All parents of the children participating in the Flanders preschool dietary survey provided their written informed consent.

3.2. Dietary intake assessment

For the current analyses, only diaries with three completed record days were included (n= 696; 66% of the collected diaries).

The fiber intake was estimated based on the Belgian NUBEL [370], the Dutch NEVO [361], and the USDA food composition databases [491], which used the enzymatic gravimetric method of the American Association of Analytical Chemists (AOAC) [83;327].

In total, 936 food items and composite dishes were encoded in the original database. All recipes that were described in depth as individual ingredients in the diaries were coded as ingredients. However, in order to classify foods easily into the food groups of the Flemish FBDG [507], eight extra composite dishes had to be disaggregated (nasi goreng, nasi goreng with egg, spaghetti Bolognese, chicken ragout, turkey ragout, lasagna, macaroni ham/cheese sauce, and stew). Spaghetti Bolognese, for instance, was disaggregated into spaghetti, noodles, minced meat, onions, tomatoes, carrots, and margarine according to the recipe list of the Flemish EPIC-soft version 2004 [124].

After the disaggregating procedures, food items were divided into 57 food groups, based on the classification of the FBDG and the expert opinion of the investigators. It should be noted that, due to lack of information, the complex food mixtures pizza (consumed by sixty-eight children during the three recorded days) and quiche (consumed by two children) were not disaggregated into their constituent components, but were categorized as a subcategory of the miscellaneous group.

In our study, we defined rest group foods (snacks and desserts) as energy-dense, low-nutritious foods based on the Flemish FBDG, considering their relatively high energy contribution (in Table 2) but low nutrient content.

3.3. Socio-economic status

SES included family situation (two-parent family, one-parent family or special situation (children living with grand-parents or others were considered as special situation)), parental employment (both parents employed, one parent employed or both parents unemployed), and level of parental education (lower secondary education, secondary education or higher education (bachelor, master or above) for both mother and father.

3.4. Statistical analysis

Descriptive statistics of the study population (mean values or frequency distributions and SD) were calculated by gender-age and gender-SES specific groups. The values of energy and DF intakes were corrected for within-person variation by means of the MSM [127]. The normality of the data and equality of the variances were tested using the Kolmogorov-Smirnov and Levene's test, respectively. The statistical differences of total energy and (energy-adjusted) DF intake between subgroups were assessed after log-transformation using the Student's *t*-test. Mean energy-adjusted daily intake from food sources was calculated based on the quartiles of total DF intake. Results were considered statistically significant at an α two-tailed level of 0.05.

The association between DF and SES was investigated by stepwise multiple linear regression analysis, by controlling for potential modifying factors (physical activity level, parental smoking, total energy intake and dietary supplement intake) and confounding factors (gender, age and nationality). Two-way interactions between potential confounding factors and SES were created and examined. In the multiple linear regression analyses the categories of higher educated mothers, higher educated fathers, unemployed parents and one-parent families were considered as references. Significance of the associations was evaluated with the T test. Outliers were removed based on residual plots.

Furthermore, to investigate the association between total or major food group-specific DF intakes (bread and cereals, potatoes and grains, vegetables, fruits, and energy-dense, low-nutritious food) and the different independent factors (maternal education level, paternal education level and parental employment), Generalized linear model (GLM) were carried out with the same references. Other covariates such as potential confounding factors (gender, age and nationality), total energy intake, dietary supplement intake, physical activity, parental smoking, and two-way interactions between SES and confounding factors and between the potential confounding factors, were included in the model.

All statistical analysis were performed using SPSS for Windows version 15.0 (SPSS Inc, Chicago, IL, USA).

4. RESULTS

4.1. Study population

A total of 661 out of 1026 children (64%) with all valid information, were included in the analysis (338 boys and 323 girls) (Table 3.2.1). Among the 365 excluded children, 330 did not complete 3-d EDR days, 51 had a missing value for gender and age, and 4 had missing gender or age.

The majority of the children (95%) were living with both parents. Approximately half of the parents had a higher education and about 70% of the children's parents were both employed.

4.2. Total energy and dietary fiber intake

The mean energy intake among preschoolers was 1455 kcal/d (849–2838 kcal/d). Boys had significantly higher energy intakes than girls. The children in the 4.0-6.5 years group had significantly higher energy intakes than the younger children ($P \leq 0.001$).

The mean total DF intake of Flemish preschoolers was 13.4 g/d (6.2–21.5 g/d) and the mean energy-adjusted DF intake was 9.3 g/1000 kcal (4.4-17.3 g/1000 kcal) (Table 3.2.1). Boys consumed significantly more DF than girls ($P < 0.001$). The elder children consumed more DF than the younger (P=0.003). However, energy-adjusted DF intake showed no significant differences between the gender-age groups.

Table 3.2.1. Anthropometric characteristics and socio-economic status (n and %), and energy and (energy-adjusted) dietary fiber intakes reported for Flemish preschool children

Characteristic	**Total**	**Boys**	**Girls**
Age (n= 661)		n (%)	
2.5-3.9 years	197 (29.8)	102 (30.2)	95 (29.4)
4.0-6.5 years	464 (70.2)	236 (69.8)	228 (70.6)
Socio-economic status			
Family situation (n= 659)			
Two-parents family	632 (95.9)	323 (96.1)	309 (95.7)
One-parent family	23 (3.5)	11 (3.3)	12 (3.7)
Special situation	4 (0.6)	2 (0.6)	2 (0.6)
Maternal education (n=655)			
Lower secondary	26 (4.0)	9 (2.7)	17 (5.3)
Secondary	250 (38.2)	127 (38.1)	123 (38.2)
Higher education	379 (57.9)	197 (59.2)	182 (56.5)
Paternal education (n=637)			
Lower secondary	49 (7.7)	25 (7.7)	24 (7.7)
Secondary	279 (43.8)	146 (45.1)	133 (42.5)
Higher education	309 (48.5)	153 (47.2)	156 (49.8)
Parental employment (n= 634)			
Both parents employed	439 (69.2)	214 (66.0)	225 (72.6)
One parent employed	157 (24.8)	88 (27.2)	69 (22.3)
Unemployed parents	38 (6.0)	22 (6.8)	16 (5.2)
		Mean intake ± SD	
Total energy intake (kcal/d)			
2.5-3.9 years*	1408.4 ± 260.4	1441.7 ± 253.0	1372.7 ± 264.9
4.0-6.5 years**	1474.4 ± 240.0[a]	1526.3 ± 233.7[b]	1420.8 ± 235.1
Energy-adjusted fiber intake (g/(1000kcal*d))			
2.5-3.9 years	9.2 ± 1.8	9.4 ± 1.8	9.0 ± 1.9
4.0-6.5 years	9.3 ± 1.9	9.3 ± 1.9	9.3 ± 1.8
Total fiber intake (g/d)			
2.5-3.9 years*	12.9 ± 3.0	13.4 ± 3.0	12.2 ± 2.9
4.0-6.5 years*	13.7 ± 3.2[b]	14.1 ± 3.3	13.2 ± 3.2[b]

SD, standard deviation

[µ] Mean daily dietary fiber intake was calculated and adjusted for within-person variation using MSM.

* Mean value was significantly different between boys and girls, Student *t*-test after log-transformation, $P < 0.05$

** Mean value was significantly different between boys and girls, Student *t*-test after log-transformation, $P \leq 0.001$

[a] Mean value was significantly different from 2.5-3.9 years old, Student *t*-test after log-transformation, $P \leq 0.001$

[b] Mean value was significantly different from 2.5-3.9 years old, Student *t*-test after log-transformation, $P < 0.05$

4.3. Food groups contributing to dietary fiber intake

The most important contributing food groups consisted of bread and cereals (29.5%), particularly bread, rolls, crackers and rice cakes, followed by fruits (17.8%, fresh fruit in particular), potatoes and grains (16.0%, potatoes in particular), energy-dense, low-nutritious foods (12.4%, sweet snacks, French fries and croquettes in particular), and vegetables (11.8%, cooked vegetables in particular) (Table 3.2.2).

Additionally, the energy-adjusted daily intakes from bread and cereals, potatoes and grains, vegetables, and fruits increased significantly among the whole population based on the quartiles of total DF intake (P<0.001, P=0.005, P< 0.001, and P< 0.001, respectively) (Table 3.2.3). Energy-adjusted intakes of the rest group, on the other hand, decreased significantly, in boys in particular (P<0.001).

Table 3.2.2. Mean and median daily intakes of food groups[µ] and their contributions to total energy and fiber intakes (n= 661)

	Food intake (g/d)			Energy		Fiber	
Food group	**Mean**	**Median**	**SD**	**%**	**order**	**%**	**order**
Beverages (including juices, excluding the rest group)	**486.2**			**5.2**		**4.8**	
Water	224.2	150.0	(226.4)	0.0		0.0	
Light beverages	23.1	0.0	(90.1)	0.0		0.0	
Tea and coffee without sugar	8.2	0.0	(43.5)	0.0		0.1	
Fruit juice	172.8	150.0	(209.3)	4.5	6	2.9	8
Vegetable juice	0.2	0.0	(6.0)	0.0		0.0	
Soup, bouillon	57.7	0.0	(101.7)	0.6		1.8	
Bread and cereals	**86.7**			**16.4**		**29.5**	
Bread, rolls, crackers, rice cakes	70.3	62.5	(46.8)	12.4	1	25.3	1
Sugared bread	7.5	0.0	(22.5)	1.7		2.2	9
Breakfast cereals (ready-to-eat, hot)	8.9	0.0	(20.0)	2.3		2.0	
Potatoes and grains	**86.7**			**5.4**		**16.0**	

Pasta, noodles	15.4	0.0	(41.0)	1.1		1.0	
Rice	6.3	0.0	(25.5)	0.6		0.9	
Potatoes	65.0	50.0	(69.3)	3.7	7	14.1	3
Vegetables	**66.5**			**1.1**		**11.8**	
Cooked vegetables	53.7	40.0	(60.1)	1.0		10.0	4
Raw vegetables	12.8	0.0	(38.3)	0.1		1.8	
Fruits (sweetened and unsweetened)	**109.9**			**4.4**		**17.8**	
Fresh fruit	94.0	68.8	(102.7)	3.6	8	15.5	2
Canned fruit	15.4	0.0	(45.4)	0.7		2.1	10
Dried fruit	0.4	0.0	(3.7)	0.1		0.2	
Olives	0.1	0.0	(1.5)	0.0		0.0	
Milk, milk products, and calcium enriched soy drinks	**439.9**			**19.9**		**6.0**	
Milk (including goat's milk)	179.0	125.0	(218.5)	6.2	4	0.0	
Flavoured milk drinks (*e.g.* Fristi, chocolate milk,…)	188.3	145.0	(226.8)	8.9	3	4.5	6
Yoghurt	4.5	0.0	(25.3)	0.2		0.0	
Sugared or aromatised yoghurt	14.2	0.0	(46.9)	0.9		0.2	
Soy drinks	15.7	0.0	(82.5)	0.6		1.0	
Milk desserts	19.9	0.0	(56.2)	1.7		0.2	
Soy-based desserts	2.3	0.0	(19.1)	0.1		0.1	
Fermented milk or soy drinks (*e.g.* actimel, yakult,…)	0.7	0.0	(7.4)	0.0		0.0	
Fresh cheese	15.3	0.0	(43.3)	1.4		0.0	
Cheese	**14.5**			**3.5**		**0.0**	
Hard cheese (no cream cheese)	11.8	0.0	(22.6)	3.0		0.0	
Cheese spread	2.7	0.0	(8.8)	0.5		0.0	
Fat, oil, cream cheese, sour cream	**8.6**			**3.3**		**0.0**	
Butter, margarine	8.3	6.0	(9.5)	3.1		0.0	

Oil	0.3	0.0	(1.4)	0.2		0.0	
Frying oil	0.0	0.0	(0.6)	0.0		0.0	
Meat, poultry, fish, eggs, vegetarian products	**90.3**			**13.5**		**1.3**	
Meat, game, meat products	37.2	20.0	(46.1)	6.0	5	0.1	
Chicken, turkey	15.9	0.0	(34.7)	1.9		0.0	
Fish, shellfish	8.5	0.0	(28.7)	0.9		0.1	
Cold cuts from meat products	20.7	6.8	(30.2)	3.5	9	0.0	
Cold cuts from fish products	0.9	0.0	(6.8)	0.2		0.0	
Eggs[†]	5.1	0.0	(18.2)	0.7		0.0	
Vegetarian products (*e.g*. tofu, tempe,…)	1.7	0.0	(11.6)	0.2		0.9	
Nuts and seeds	0.3	0.0	(3.4)	0.1		0.2	
Rest group (snacks and desserts)[a]	**201.8**			**26.8**		**12.4**	
Brioches	3.5	0.0	(17.0)	0.8		0.6	
Sweet snacks	43.6	32.0	(43.5)	11.9	2	5.2	5
Salty snacks	2.1	0.0	(9.8)	0.8		0.9	
Tea and coffee with sugar	3.2	0.0	(26.6)	0.0		0.0	
Soft drinks	97.7	0.0	(169.4)	2.7		0.0	
Salty sauces	12.5	0.0	(24.9)	1.6		0.6	
Cream	0.3	0.0	(2.6)	0.1		0.0	
Sweet sauces	0.1	0.0	(2.5)	0.0		0.0	
Chocolate	3.1	0.0	(9.5)	1.1		0.2	
Chocolate spread	9.4	0.0	(13.9)	3.5	10	1.1	
Other sweet spread (*e.g*. jam, honey, …)	5.3	0.0	(11.6)	1.0		0.3	
Sugar	0.1	0.0	(0.9)	0.0		0.0	
Fried snacks	0.1	0.0	(2.6)	0.0		0.0	
French fries, croquettes	14.6	0.0	(37.7)	2.6		3.5	7

Sweet desserts (*e.g.* ice cream, tiramisu, …)	6.2	0.0	(23.2)	0.8	0.1
Miscellaneous	**4.2**			**0.5**	**0.3**
Pizza and quiches	2.2	0.0	(17.8)	0.3	0.2
Other miscellaneous‡	2.0	0.0	(21.3)	0.2	0.1

µ These mean food group intakes are rough estimates calculated from the raw data on which these nutrient contributions are based, without adjustment for within-person variation. The high number of non-consumers in some food groups hindered the adjustment for within-person variation.

†Includes only eggs reported separately and eggs included in disaggregated food mixtures

‡Includes foods or components with negligible contributions to the total nutrient intakes that could not be categorized in the above food groups (*e.g.* herbs and spices, monosodium glutamate, starch, plain gelatine, artificial sweeteners, pectin, cocoa powder,…)

[a] Rest group (snacks and desserts) was defined as energy-dense, low-nutritious foods.

Table 3.2.3. Mean energy-adjusted daily intakes (g/d) from the main food groups contributing to dietary fiber intake for the children assigned to the diffe intake quartiles (n= 661)

	Fiber intake quartiles												P†		
	Total				Boys				Girls						
Food groups µ	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Total	Boys	Girls
Bread and cereals	51.6	57.5	62.6	66.7	53.0	57.6	61.9	68.0	50.6	57.4	63.7	64.7	<0.001	<0.001	<0.001
Potatoes and grains	53.5	59.1	63.3	64.1	59.1	59.9	63.4	57.3	49.2	58.5	63.3	74.8	0.005	0.725	<0.001
Vegetables	33.1	42.3	47.0	60.1	33.2	42.5	46.1	56.4	33.0	42.1	48.2	65.9	<0.001	<0.001	<0.001
Fruits	41.2	63.3	83.2	110.4	33.5	54.0	80.7	106.6	47.3	70.4	86.4	116.3	<0.001	<0.001	<0.001
Restgroup‡	123.3	112.5	93.5	82.4	143.2	140.7	89.7	87.8	107.9	90.8	98.3	73.9	<0.001	<0.001	0.178

µ Quartiles based on total fiber intake among Flemish children.

†Statistical analysis was tested by ANOVA (bread and cereals, potatoes and grains) and Kruskal-Wallis (vegetables, fruits and restgroup) test.

‡ Rest group (snacks and desserts) was defined as energy-dense, low-nutritious foods.

4.4. Association between total and food-group specific dietary fiber intakes and socio-economic status factor

A significant positive association was observed between children's total DF intake and one-employed–parent-families (B= 0.580, *P*= 0.019), compared to families where both parents were unemployed, whereas a negative association was found with secondary maternal education (B = -0.634, *P*= 0.004), as opposed to higher maternal education (Table 3.2.4). The intake of total fiber among Flemish preschoolers depended on preschoolers' age and family situation. With getting 1 year older, preschoolers from two-parent-family consumed more 0.212 g fiber per day than those preschoolers from one-parent family.

Table 3.2.4. Stepwise multiple linear regression analysis of the potential association between total DF intake and socio-economic status among Flemish preschoolers (n= 661)

Total fiber intake †‡	B	**SE**	**95% CI**		***P***
Secondary maternal education $^{\mu}$	-0.634	0.219	-1.1	-0.203	0.004
One employed parent $^{\mu}$	0.580	0.247	0.095	1.1	0.019
Age* two-parents family $^{\mu}$	0.212	0.097	0.021	0.403	0.030

SE, standard error of B coefficient; CI, confidence interval
$^{\mu}$ Higher maternal education, and two-unemployed–parent-families and one-parent families were used as reference categories.
†Adjusted for total energy intake, age, gender, nationality, and children's level of physical activity, parental lifestyle and interactions.
‡Non-significant variables with coefficients B
Higher maternal education: B = 0.125, *P*= 0.148; Secondary paternal education: B = -0.037, *P*= 0.297; Higher paternal education: B = 0.038, *P*= 0.303; Both employed parent: B = -0.040, *P*= 0.547; Two-parents family: B = 0.010, *P*= 0.797

GLM was used to investigate associations between DF intake from main food sources, and SES (Table 3.2.5). Compared to children of higher educated mothers, those with a lower secondary maternal education had lower bread and cereal- and rest group, but higher potato and grain-derived fiber intakes (B = -8.4, *P*= 0.009, B = -4.3, *P*= 0.001, B = 8.8, *P*< 0.001, respectively). Conversely, preschoolers of fathers with a secondary education consumed more bread and cereal-, and fruit-derived fibers (B = 3.0, *P*= 0.027, B = 2.9, *P*= 0.036, respectively) than those with a higher paternal education, whereas children of lower secondary educated fathers had lower potato and grain-derived fiber intakes (B = -4.0, *P*= 0.026).

Furthermore, preschoolers' intake of fiber derived from energy-dense, low-nutritious foods were higher in two-parents families than in one-parent families ($B = 3.1$, $P = 0.016$). Children with one or both parents being employed consumed less fibers derived from energy-dense, low-nutritious foods as compared to preschoolers of unemployed parents ($B = -2.8$, $P = 0.010$, $B = -2.6$, $P = 0.012$, respectively).

Table 3.2.5. Generalized linear model of the potential association between food group-specific fiber intake and socio-economic status of Flemish preschoolers (n= 661)

	Bread and cereals			Potatoes and grains			Vegetables			Fruits			Rest group†		
Independent variables	B (SE)	95% CI	*P*	B (SE)	95% CI	*P*	B (SE)	95% CI	*P*	B (SE)	95% CI	*P*	B (SE)	95% CI	*P*
Maternal education															
Lower secondary	-8.4 (3.2)	-14.7, -2.1	0.009	8.8 (2.2)	4.4, 13.1	<0.001	2.5 (2.2)	-1.9, 6.8	0.262	-1.7 (3.3)	-8.2, 4.8	0.613	-4.3 (1.3)	-7.0, -1.7	0.001
Secondary	-2.2 (1.4)	-4.9, -0.42	0.098	0.16 (0.94)	-2.0, 1.7	0.862	-0.016 (0.94)	-1.9, 1.8	0.974	-1.9 (1.4)	-4.6, 0.88	0.183	0.082 (0.57)	-1.0, 1.2	0.885
Paternal education															
Lower secondary	3.9 (2.6)	-1.2, 8.9	0.131	-4.0 (1.8)	-7.5, -0.48	0.026	-0.41 (1.8)	-3.9, 3.1	0.819	-1.2 (2.7)	-6.5,4.0	0.640	1.5 (1.1)	-0.61, 3.7	0.160
Secondary	3.0 (1.3)	0.33, 5.6	0.027	-1.0 (0.94)	-2.8, 0.82	0.862	0.39 (0.93)	-1.4, 2.2	0.676	2.9 (1.4)	0.20, 5.7	0.036	0.73 (0.57)	-0.39, 1.8	0.201
Family situation															
Two-parents	2.3 (3.0)	-3.7, 8.2	0.439	1.9 (2.1)	-2.2, 5.9	0.372	1.8 (2.1)	-2.3, 5.9	0.392	2.3 (3.1)	-3.8, 8.4	0.464	3.1 (1.3)	0.58, 5.5	0.016
Parental employment															
Both employed	-1.4 (2.4)	-6.1, 3.4	0.577	-0.89 (1.7)	-4.2, 2.4	0.600	1.4 (1.7)	-2.0, 4.7	0.424	-2.9 (2.5)	-7.8, 2.1	0.254	-2.6 (1.0)	-4.6, -0.56	0.012
One employed	-1.2 (2.6)	-6.3, 3.9	0.653	-0.061 (1.8)	-3.6, 3.5	0.973	2.0 (1.8)	-1.6, 5.5	0.272	-2.3 (2.7)	-7.5, 3.0	0.399	-2.8 (1.1)	-5.0, -0.67	0.010

SE, standard error of B coefficient, CI, confidence interval

µ Unemployed parents, higher educated parents and one-parent family were as reference

† Rest group (snacks and desserts) was defined as energy-dense, low-nutritious foods.

5. DISCUSSION

5.1. Total and food group-specific fiber intake

In this food consumption survey among Belgian preschoolers, the DF intake was on average 13.4 g/d (boys: 13.9 g/d, girls: 12.9 g/d; $P< 0.001$) and the mean energy-adjusted fiber intake 9.3 g/(1000 kcal*d) (boys: 9.2 g/(1000 kcal*d), girls: 9.3 g/(1000 kcal*d); $P= 0.748$). It is noteworthy that a higher energy intake seems to correspond with a higher DF intake in boys, possibly due to a higher overall dietary intake. The mean DF intake among Flemish preschool children did not reach the requirements proposed by the BSHC, especially not for the children aged 4-6.5 years, with 90% of the boys and 93% of the girls not meeting the guidelines.

Compared to the recent small-scale Flemish study of Bosscher *et al.* (2002) (2-3 years old children: 10 g/d based on 7 d-dietary records, n= 115), the DF intakes reported in the present study were higher [71]. As limited data is available on Belgian preschoolers fiber intake, additional comparisons were made with preschoolers from other countries with comparable age to evaluated our results. The DF intakes among Belgian preschoolers were similar to those among European children in general [284], and German (10.3-16.2 g/d) [85] and Italian (11.1-14.6 g/d) [190] children in particular, all assessed by the same dietary assessment method (food diaries). Conversely, the DF intakes among Belgian preschoolers were higher than those reported for Spanish (boys: 11.2 g/d, girls: 10.1 g/d) [431] and American children (9.1-13.1 g/d) [6], and lower than those of Swiss children (14.8-16.9 g/d), all based on two 24-h recalls.

Furthermore, this study aimed to identify the most important contributors to total DF intake among preschoolers. However, differences in dietary assessment and, in particular, classification of food items into food groups, often hamper sound comparisons between different study populations. Nevertheless, in general, the main sources of DF were similar for the current study population and children living in Antwerp [71]. However, the latter study reported lower contributions for cereals and pastry (6.6%), fruit (15.1%), and potatoes (14.5%). On the other hand, vegetables (13.9%), soup (8.0%), and sugar and candy products (2.1%) contributed more to the total DF intake than in the more general and representative study population of Flemish preschoolers involved in the present study. Additionally, we found that the group of bread and cereals was the most important contributor of DF, as also observed among American children [420]. Although the contributions of bread and cereals, and vegetables were in line with US reports (29.4% and 11.3%, respectively), potatoes and fruits contributed more to the DF intakes of Belgian preschoolers than of American children (11.2% and 13.1%, respectively) [420]. In comparison to Spanish children [415], the contributions of bread and cereals, potatoes, and vegetables were lower in Spanish children (11.2%, 4.3%, and 7.9%, respectively) than in ours, while those of fruits and legumes were much higher in Spanish children (25.6% and 20.1%, respectively) than in Belgian children. Finally, the average DF intakes from cereals, fruits, and vegetables were substantially lower in Belgian than German children (4.4-8.0 g/d, 2.8-3.3 g/d, and 2.4-3.0 g/d, respectively) [85].

When looking at the food groups that are being under-consumed according to the FBDG [232] and taking into account the contributions of these foods to the total fiber intakes in these preschoolers, it can be concluded that higher intakes of whole-wheat bread, fruits, and vegetables could importantly increase the fiber intakes and should, therefore, be promoted among preschoolers.

5.2. Associations of fiber intake with socio-economic status

To the best of our knowledge, there is no data available on possible associations between DF intake and SES factors among Belgian children. Our results indicate that children of secondary educated mothers have lower DF intakes than those of higher educated mothers, whereas children with one parent being employed consumed more DF compared to those with unemployed parents. Similarly, maternal and paternal level of education were related to the food group-specific fiber intakes of their children, with lower bread and cereal-, higher potato and grain–, and lower energy-dense, low-nutritious foods-derived fiber intakes reported for children of lower secondary educated mothers compared to those of higher educated mothers. On the other hand, higher bread and cereal-, and fruit-derived fiber intakes were observed with paternal secondary education as opposed to higher education. Additionally, children with employed parents had higher total DF intakes, but consumed less DF from energy-dense, low-nutritious food than preschoolers with both parents unemployed. In two-parent families, children had higher intakes of energy-dense, low-nutritious food-derived fibers than in one-parent families.

Perry *et al.* (1988) suggested that parental involvement plays a critical role in promoting children's health behavior and dietary habits at early age [394]. Parental involvement might result in consumption of fiber from high-nutritious foods (vegetables and fruit). In the present study, DF intakes, more from high-nutritious foods (vegetables and fruit) and less from energy-dense, low-nutritious foods, were reported for preschoolers of higher educated mothers. Also, evidence showed that children in low SES families were found to have higher total energy, cholesterol, and fat intakes and lower vegetable and fruit intakes [88;138;139;269;286]. Moreover, children of unemployed parents or less income families consumed unhealthier DF derived from energy-dense, low nutritious foods. The cost of healthy food, reduced food choices, and lack of education in low SES families might lead to lower vegetable- and fruit-derived fiber intakes and, consequently, a higher prevalence of children at risk to become OW or obese, and to develop chronic diseases [135;226;536]. Children with both employed parents, however, had less DF intake than those with one-employed parent in our study, which might be influenced by less parent's free time.

We observed that dietary sources from vegetables and fruit contributing to DF intake in our study were much less compared to other food sources based on the quartiles of total fiber intake. Although vegetables and fruit were ranked second and fifth in DF contribution, children had extremely lower DF intake from raw vegetables (1.8%) compared to cooked ones (10.0%). In addition, in our finding, children of higher educated mothers and secondary fathers and those with one or both parents being employed had more DF intake from vegetables and fruit, which indicates that lower secondary educated and unemployed families need more attention during health promotion campaigns. Our results also suggest that the level of maternal education is more indicative for dietary habits of their preschool aged children than the level of paternal education.

5.3. Strengths and limitations

The present study was the first food consumption survey among preschoolers comparing the associations between total and food group-specific DF intakes, and SES in Belgium while covering the whole Flemish region. Therefore, the results of this large cross-sectional study represent the Flemish preschool children's dietary habits with a good representativity compared to the more local and small-scale surveys that were executed before.

Like all studies, some limitations should be taken into consideration. First, this study suffered from some selection bias, with the lower SES group being underrepresented [229], which might have influenced the true DF intake and the linear associations.

Furthermore some limitations regarding the dietary assessment method are noteworthy. No dietary assessment method is perfect and every method is prone to some degree of misreporting. The method of 3-d EDR reflects the individual children's short-term rather than usual intakes. However, we corrected for within-person variability by using the MSM method to get more precise individual usual daily DF intakes. The percentage of under-reporters, excluded in this study, in the final sample for analysis was very low (2%). In addition, a relative validation study was conducted in which the results derived from a FFQ were compared with those derived from our 3-d EDR for calcium intake, food intake and for a diet quality index [227;230;231].

Then, the decisions regarding the food grouping were based on the Flemish FBDG and on the judgment of the investigators, which might have implications for the findings. The food composition of fortified foods, highly consumed by Flemish preschoolers, was rather hard to define and, in some cases, information from the industry or from packing materials had to be used. Furthermore, the definition of DF in Belgium is considered as carbohydrates with three to ten monomeric units, which might result in differences with international recommendations [220]. Also no real information is available on the low molecular weight DF fraction and limitations of AOAC methods used [109;400]. Our dataset was not adjusted for possible alterations in fiber content or quality due to food processing, which may have attenuated the accuracy of our total DF estimates.

Moreover, seasonal variation was impossible to avoid completely in this study, although all days of the week were covered in our dietary survey. However, low seasonal variations influencing dietary intakes in Belgian population was reported because the widespread availability of most foods all year round [124].

6. CONCLUSION

Our results showed that the mean total DF intake among preschoolers is below the guidelines of the BSHC, especially for the children aged 4-6.5 years. Girls ingested significantly less fibers than boys. The most important contributor to the total DF intake was the group of bread and cereals, followed by fruit, potatoes, energy-dense, low nutritious foods, and vegetables. Maternal education level and parental employment were significantly associated with DF intake. Overall, DF intakes from high-nutritious foods (vegetables and fruit) were higher in preschoolers of higher educated mothers and those with one or both parents being employed. These findings suggest that dietary fiber should be promoted in general and low SES families should be addressed in particular.

CHAPTER 4

RESULTS FROM THE BELGIAN POPULATION

CHAPTER 4.1

Plant and animal protein intake and its association to overweight and obesity among the Belgian population

Chapter based on this manuscript:

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1. ABSTRACT

Objective

The present study was to assess animal and plant protein intakes in the Belgian population and to examine their relationship with overweight and obesity.

Methods

The subjects participated in the Belgian National Food Consumption Survey conducted in 2004. Food consumption was assessed by using two non-consecutive 24-h dietary recalls.

Results

About 3083 participants (≥15 years of age; 1546 males, 1537 females) provided completed dietary information. Animal protein intake (47 g/d) contributed more to total protein intakes of 72 g/d than plant protein intake, which accounted for 25 g/d. Meat and meat products were the main contributors to total animal protein intakes (53%), whereas cereals and cereal products contributed most to plant protein intake (54%). Males had higher animal and plant protein intakes than females (P<0.001). Legume and soya protein intakes were low in the whole population (0.101 and 0.174 g/d, respectively). In males, animal protein intake was positively associated with BMI (β = 0.013; P=0.001) and waist circumference (WC; β = 0.041; P=0.002). Both in males and females, plant protein intake was inversely associated with BMI (males: β=-0.036; P<0.001; females: β=-0.046; P=0.001) and WC (male: β=-0.137, P<0.001; female: β=-0.096, P=0.024).

Conclusion

Plant protein intakes were lower than animal protein intakes among a representative sample of the Belgian population and decreased with age. Associations with anthropometric data indicated that plant proteins could offer a protective effect in the prevention of overweight and OB in the Belgian population.

2. INTRODCUTION

In recent decades, intakes of dietary protein have been associated with treating chronic diseases such as OB and CVD besides improving health outcomes [167;538]. Evidence indicates that a high dietary protein intake decreases the risk of NCD via the regulation of energy intake, increment of satiety, lowering of SBP and DBP, decrement of TC levels, and presence of LCL-C and TG [2;22;68]. In addition, high protein intakes are associated with the prevention of the development of chronic diseases, including OB, MetS, CVD, T2D, osteoporosis, and breast and prostate cancer [2;129;320;480;485;528].

Findings from recent randomized controlled trials relate plant proteins to health benefits more than animal proteins [14;27;87;243;246;248;299;366;555], mainly due to factors affecting the level of hypercholesterolaemic amino acids present in plant proteins [274]. However, the debate on the potential health effects of animal protein- and plant protein-rich diets is still ongoing. For instance, some studies have reported a positive association between animal protein intakes and the risk of chronic diseases [12;145;293;441], whereas others have indicated an inverse relationship [21;159;378]. One of these trials, involving healthy menopausal women, has suggested that milk whey protein can prevent bone loss [21], while two others trials with OW or insulin-resistant subjects have indicated that proteins from meat, poultry, fish and dairy foods had beneficial metabolic effects [159;378], and improved insulin sensitivity [159;378].

In Belgium, information on plant and animal protein intakes of the population is still lacking until now. Therefore, the present study aims (1) to estimate the intake levels of animal and plant proteins in a representative sample of the Belgian population and (2) to examine their associations with OW and OB measured by BMI and WC.

3. METHODS

3.1. Study design and data collection

The BNFCS [123] was performed in 2004 following largely the recommendations of the EFCOSUM [79]. More details on the survey can be found else-where [123]. Belgian national citizens aged 15 years or older, residing in private households in Belgium, were eligible to participate in the national survey. The population was stratified by sex and in four age groups (15–18, 19–59, 60–74 and ≥75 years). Approximately 400 individuals were allocated in each sex–age group. Participants were selected from the national register using a multi-stage stratified sampling procedure. Institutionalized individuals, not able to speak one of the national languages or physically or mentally unable to be interviewed, were excluded from the survey. In total, 7543 individuals were invited to participate.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and approved by the medical ethical committee of the Scientific Institute of Public Health, Brussels. Written or verbal informed consent was obtained from all subjects. Verbal consent was witnessed and formally recorded.

3.2. Dietary intake assessment

Two repeated, non-consecutive 24 h dietary recall interviews were used to collect information on each participant's food consumption. The first 24-h recall was obtained through a computer-assisted personal interview during a home visit by a trained dietitian. The second 24-h recall was performed 2–8 weeks later during a second home visit (median 3 weeks). Interviews were randomly allocated to different days of the week and over a 12-month period in an effort to reduce within-person variation and to avoid seasonality effects. The 24-h recalls collected information on the types and quantities of foods and beverages consumed over the preceding day to the interview.

The dietitians used European Prospective Investigation into Cancer and Nutrition software (EPIC-SOFT; International Agency for Research on Cancer (IARC), Lyon, France) to obtain standardized 24 h recall interviews. EPIC-SOFT was designed to obtain a detailed description and quantification of all foods and beverages consumed in a standardized way [451].

Quantification was facilitated using a picture book with colored photographs describing foods of different portion sizes [123].

Animal and plant protein contents were estimated using the Belgian food composition table NUBEL [370], the Dutch food composition database NEVO [361] and the USDA food composition guidelines [490]. In the present study, consumption of soya products was analyzed separately from the legumes food group because of their potential health effects. The US Food and Drug Administration [166] approved that a daily consumption of soya protein can prevent chronic diseases.

In the present study, four and six main food groups, respectively, contributed most to the animal and plant protein intakes. The four main food groups contributing to the levels of animal protein intake were dairy products, meat and meat products, fish and shellfish, and eggs and egg products. Dairy products included milk, milk beverages (including cream desserts and puddings (milk-based), dairy and non-dairy creams, milk for coffee, and creamers), yogurt, fromage blanc and petits suisses and cheeses (including fresh cheeses). The group of meat and meat products included fresh meat (beef, veal, pork and lamb), poultry and game (chicken, turkey, duck and rabbit), and processed meat, whereas the group of fish and shellfish represented all fish, crustaceans, molluscs, fish products and fish in crumbs. Eggs were the most important item in the egg and egg products group.

Plant proteins were derived mainly from potatoes and other tubers, vegetables, legumes, fruits, nuts and seeds, cereal and cereal products, and soya products. The group of potatoes and other tubers consisted mainly of potatoes. Vegetables included leafy vegetables, fruiting vegetables such as tomato and pumpkin, root vegetables, cabbages, mushrooms, grain and pod vegetables, onions, garlic, stalk vegetables and sprouts, mixed salad and mixed vegetables. Soybeans and derived products were excluded from the legumes group and were classified as a separate group. Fruits referred to all fruits, including fresh fruits (fruits, mixed fruits and olives) and nuts and seeds. Cereals and cereal products included mainly flour, flakes, starches, semolina, pasta, rice, other grains, breakfast cereals, bread, crisp bread, rusks, salty biscuits, aperitif biscuits and dough and pastry.

3.3. Anthropometric measurements

Weight (kg) and height (m) were self-reported. WC was measured by a trained dietitian at home while participants were standing upright (upper clothes were raised to enable measurement of WC on the skin or underwear). Pregnant women reported pre-gestational weights. BMI was calculated as weight (kg)/height (m^2). Adult participants were allocated to four BMI categories according to the cut-off criteria of the WHO [543] for adult BMI: underweight (<18.5 kg/m²); normal-weight (18.5 - 24.9 kg/m²); OW (25.0 - 29.9 kg/m²); and obese (≥30.0 kg/m²). Adolescent participants were classified into four similar BMI categories based on the Flemish cut-off values [512] for underweight. Cut-off points for normal-weight, OW and obese were based on the criteria proposed by Cole *et al.* [102]. For adult WC, sex-specific cut-off criteria were used [290]. For males, <94 cm was defined as normal, 94–102 cm as normal to borderline, ≥102 cm as high risk of OW and obese (referred to as 'too large' in Table 1). For females, <80 cm was defined as normal, 80–88 cm as normal to borderline, ≥88 cm as high risk of OW and obese. The cut-off criteria of adolescent WC were based on Taylor *et al.* [475].

3.4. Statistical analysis

The Statistical Package for the Social Sciences for Windows version 15 (SPSS, Inc., Chicago, IL, USA) was used to perform descriptive and statistical analyses. Descriptive statistics are presented in the sex–age-specific groups as means with their standard errors. Total energy, total protein, animal and plant protein intake and percentage of energy intake (Table 4.1.2) were normally distributed, whereas animal and plant protein intakes from food groups (Tables 4.1.3 and 4.1.4, respectively) were skewed. Student's *t* test, ANOVA with Bonferroni correction and the Mann–Whitney U test were used to examine statistically significant differences, with a two-tailed significance level set at 0.05.

Multiple linear regression analysis (Generalized linear model (GLM)) by the sex–age strata was used to evaluate the association between BMI, WC and animal and plant protein intakes. Each model included BMI and WC as separate dependent variables, animal and plant protein as covariates and age as the factor variable. Interactions were tested, and the significance level was estimated by type 3 Wald X^2 test.

4. RESULTS

Individuals who provided two 24-h dietary recall interviews with valid information were included in the analysis (3083 out of a total of 7543). Male (n=1546) participants had a mean of 25 kg/m^2 for BMI and a mean of 88 cm for WC. In total, 34% of the males were defined as OW, 10.1% as obese and 29% had a too large WC. Mean BMI for female (n=1537) participants was 24 kg/m^2, and mean WC was 80 cm. In total, 25% of the females were defined as OW, 10.5% as obese and 42% had a too large WC (Table 4.1.1).

Most of the participants in the older categories were categorized as OW or obese (60–75 years: 63%; ≥75 years: 50%) and with borderline or too large WC (60–75 years: 80%; ≥75 years: 81%).

4.1. Total, animal and plant protein intakes

Total protein intakes (1.2 MJ/d) contributed 15.4% to the total energy intakes of the population. Animal protein intakes contributed most and delivered a mean energy intake of 0.795 MJ/d. Animal protein intake (47 g/d, range 0.030–222 g/d) was the main contributor (64 %) to the total protein intakes (mean 72 g/d), while plant protein intake accounted for 25 g/d (range 2.4–83 g/d). The total protein intakes of the present study population were in line with the WHO/FAO/United Nations University recommendations (i.e. 10.0–15.0% of the total energy intake) [551] (data not shown).

Total protein, animal protein and plant protein intakes were significantly higher in males than in females (Table 4.1.2). Percentage energy contributions from the total protein and animal protein intakes were significantly lower in male and in female adolescents than in the older age groups. The contribution of plant proteins to the total energy intakes was higher in elderly males aged ≥75 years and lowest in females aged ≥75 years.

When examined by sex, total protein intakes were higher among adults (19–59 years) and lower among the elderly population ≥75 years. Adult males (19–59 years) reported significantly higher animal protein intakes, while elderly males (≥75 years) had the lowest. For female participants, on the other hand, animal protein intakes in the age groups of 19–59 years and 60–74 years were significantly higher than those in the other age groups. Plant protein intakes decreased with age in both sex groups, resulting in significant differences between the youngest and the oldest age groups (P<0.001 for both).

Table 4.1.1. BMI and waist circumference measurements of subjects participating in the Belgian National Food Consumption Survey (Mean values with their standard errors, n=3083)

		BMI*						WC*				
	n	Mean (kg/m^2)	SEM	Under-weight (%)	Normal-weight (%)	Over-weight (%)	Obese (%)	Mean (cm)	SEM	Normal (%)	Border-line (%)	Too large (%)
Gender												
Males	1546	25	0.1	3.1	52	34	10.1	88	0.7	43	27	29
Females	1537	24	0.1	5.7	58	25	10.5	80	0.7	39	19	42
Age group												
15-18	762	21	0.1	9.7	79	10.2	1.3	76	0.6	72	20	7.6
19-59	828	24	0.2	3.7	60	26	10.1	81	1.0	51	24	25
60-75	789	26	0.2	1.0	36	44	18.6	91	0.9	20	27	53
≥75	704	25	0.2	3.2	46	40	10.8	90	1.2	19	22	59

BMI, Body Mass Index; WC, Waist circumference; SEM**,** standard error of the mean

* Weighted mean of BMI, WC

Table 4.1.2. Total energy, total protein, animal and plant protein intake, and percentage of energy intakes of the survey participants (Mean values with their standard errors, n=3083)

	Age group								
	15-18		19-59		60-74		≥75		P †
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Total energy intake (MJ/d)**									
Males	10.5[a]	39	10.3[a]	45	9.0[b]	32	8.2[c]	31	<0.001
Females	7.5 [a]	27	6.9[b]	26	6.6[b]	25	6.2[c]	23	<0.001
Total protein (g/d)**									
Males	85[a]	1.4	91[b]	1.6	82[a]	1.2	74[c]	1.1	<0.001
Females	61[a]	0.9	64[a]	1.0	62[a]	1.0	59[b]	1.1	0.002
Animal protein (g/d)**									
Males	52[a]	1.2	61[b]	1.4	55[a]	1.0	49[c]	0.9	<0.001
Females	37[a]	0.8	42[b]	0.8	42[b]	0.8	40[a]	0.9	<0.001
Plant protein (g/d)**									
Males	30[a]	0.6	30[a]	0.6	27[b]	0.5	25[c]	0.5	<0.001
Females	24[a]	0.4	22[b]	0.4	21[c]	0.4	18.8[d]	0.4	<0.001
				Energy intake (%)					
Total protein									
Males	13.8[a]	0.2	15.2[b]	0.2	15.7[b]	0.2	15.5[b]	0.2	<0.001
Females	14.0[a]	0.2	16.1[b,*]	0.2	16.3[b, *]	0.2	16.4 [b, *]	0.3	<0.001
Animal protein									
Males	8.8[a]	0.2	10.2[b]	0.2	10.5[b]	0.2	10.3[b]	0.2	<0.001
Females	8.5[a]	0.2	10.6[b]	0.2	10.9[b]	0.2	11.2[b, **]	0.2	<0.001
Plant protein									
Males	4.9[a]	0.1	5.1[a]	0.1	5.2[a]	0.1	5.3[b]	0.1	0.014
Females	5.4[a]	0.1	5.6[a, **]	0.1	5.4[a, **]	0.1	5.2[b]	0.1	0.004

SEM, standard error of the mean

[a,b,c,d] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$, ANOVA with Bonferroni correction).

Mean values were significantly different between men and women: * $P< 0.05$, ** $P< 0.001$ (Student's *t*-test).

† P value for mean differences between gender-age groups (ANOVA)

4.2. Main food groups

Tables 4.1.3 and 4.1.4 show, respectively, the food groups contributing 57% to the total animal protein intakes (dairy products, meat and meat products, fish and shellfish, and egg and egg products) and 28% to the total plant protein intakes (potatoes and other tubers, vegetables, legumes (excluding soya products), soya products, fruits, and cereal and cereal products). Meat protein was the main contributor to the total protein intakes (34%), with a mean intake of 26 g/d, followed by cereal protein (19.3%), with a mean intake of 13.7 g/d, and dairy protein (15.1%), with a mean intake of 11.0 g/d (data not shown).

For both sexes, meat and meat products contributed most to the total animal protein intakes (males: 55%, mean intake of 31 g/d; females: 50%, mean intake of 21 g/d; $P<0.001$), followed by dairy products (males: 22 %, mean intake of 11.9 g/d; females: 26%, mean intake of 10.0 g/d; $P<0{\cdot}001$) (data not shown). Compared with males, females consumed less meat and dairy proteins derived from the above-mentioned food groups in general and their specific subgroups, except for yogurt. In particular, proteins from fresh and processed meat were consumed significantly less by females in all age groups (range of consumption: males and females, respectively: 14.3–18.2 and 10.2–11.6 g/d for fresh meat; 7.3–9.2 and 3.9–5.1 g/d for processed meat; $P<0.001$ for both).

The elderly population (60–74 or ≥75 years) consumed less proteins derived from dairy and meat products compared with the other age groups. Female adolescents had significantly lower meat protein intakes than others (18.9 g/d contributing to 30% of the total animal protein intake). The elderly population (60–74 years) reported the lowest and the highest fish and shellfish protein intakes (males: 7.9%, mean intake of 6.4 g/d; females: 6.7%, mean intake of 4.4 g/d). Protein intakes from eggs and egg products were not significantly different between the sex–age groups, with the exception of elderly females (≥75 years) who had the lowest consumption among the sample.

Cereals and cereal products (males: 55 %, mean intake of 15.9 g/d; females: 52 %, mean intake of 11.4 g/d) contributed most to the total plant protein intakes followed by potatoes and other tubers, vegetables and fruits. The consumption of soya proteins was very low (0.174 g/d). Intakes from potatoes and other tubers, vegetables and fresh fruits were significantly higher in the elderly population (60–74 and ≥75 year groups) than in the adolescent and adult groups. The latter groups consumed, however, significantly more proteins from cereals and cereal products (data not shown)

Table 4.1.3. Mean total animal protein intakes and intakes from main sources stratified by age (years) and sex

(Mean values with their standard errors, n=3083)

Animal protein food sources	Contribution to total protein intake (%)*				Mean animal protein intake (g/d)*				$P^{\dagger}$
	Males		Females		Males		Females		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
TOTAL ANIMAL PROTEIN									
15-18	56[a]	0.7	53[a]	0.7	49[a]	1.2	33[a]	0.8	<0.001
19-59	58[a]	0.7	57[b]	0.6	54[b]	1.3	37[b]	0.8	<0.001
60-74	60[b]	0.6	58[b]	0.6	49[a]	1.0	37[b]	0.8	<0.001
≥75	58[a]	0.6	58[b]	0.7	43[c]	0.9	35[a]	0.8	<0.001
DAIRY PRODUCTS									
15-18	15.4[a]	0.6	16.8[a]	0.6	13.5[a]	0.6	10.4[a]	0.4	<0.001
19-59	14.7[a]	0.6	17.2[a]	0.5	13.4[a]	0.6	11.1[a]	0.4	0.027
60-74	13.0[b]	0.5	14.6[b]	0.5	10.8[b]	0.5	9.3[b]	0.4	0.112
≥75	13.3[b]	0.5	15.1[b]	0.6	9.8[b]	0.4	8.9[b]	0.4	0.256
Milk									
15-18	6.2[a]	0.4	6.0[a]	0.4	5.6[a]	0.4	3.7[a]	0.2	<0.001
19-59	3.1[b]	0.2	4.1[b]	0.3	2.8[b]	0.2	2.7[b]	0.2	0.841
60-74	2.6[b]	0.2	2.9[c]	0.2	2.1[b]	0.2	1.8[c]	0.1	0.525
≥75	3.3[c]	0.2	3.8[d]	0.3	2.4[b]	0.2	2.2[c]	0.2	0.454
Yogurt									
15-18	1.3[a]	0.2	1.8[a]	0.2	1.1[a]	0.1	1.1[a]	0.1	0.058
19-59	1.9[b]	0.2	3.1[b]	0.2	1.7[b]	0.2	2.1[b]	0.2	0.018
60-74	1.5[b]	0.2	3.2[b]	0.3	1.4[a]	0.2	2.1[b]	0.2	<0.001

≥75	1.7[b]	0.2	2.4[a]	0.2	1.3[a]	0.1	1.5[c]	0.2	0.173
Cheeses									
15-18	7.9[a]	0.4	9.1[a]	0.5	6.7[a]	0.4	5.6[a]	0.3	0.160
19-59	9.7[b]	0.5	10.1[b]	0.4	8.9[b]	0.5	6.5[b]	0.3	0.029
60-74	8.9[a]	0.5	8.8[a]	0.4	7.4[a]	0.4	5.5[a]	0.3	0.006
≥75	8.3[b]	0.4	8.9[a]	0.5	6.1[a]	0.4	5.2[a]	0.3	0.166
MEAT AND MEAT PRODUCTS									
15-18	36[a]	0.9	30[a]	0.9	31[a]	1.1	18.9[a]	0.7	<0.001
19-59	36[a]	0.9	32[a]	0.8	34[a]	1.1	21[b]	0.8	<0.001
60-74	37[a]	0.9	34[b]	0.9	31[a]	0.9	22[b]	0.7	<0.001
≥75	36[a]	0.9	36[c]	1.0	27[b]	0.8	22[b]	0.7	<0.001
Fresh Meat (beef, veal, pork, lamb)									
15-18	19.2[a]	0.8	15.9[a]	0.9	16.6[a]	0.8	10.2[a]	0.6	<0.001
19-59	19.3[a]	0.8	16.1[a]	0.8	18.2[a]	0.9	10.7[a]	0.6	<0.001
60-74	19.6[a]	0.8	17.9[a]	0.8	16.2[a]	0.7	11.4[a]	0.5	<0.001
≥75	19.1[a]	0.8	19.3[b]	0.9	14.3[b]	0.7	11.6[b]	0.6	0.029
Poultry (chicken, duck, rabbit, game)									
15-18	8.1[a]	0.6	7.5[a]	0.6	7.6[a]	0.7	4.8[a]	0.4	0.086
19-59	7.0[a]	0.6	8.1[a]	0.6	6.6[a]	0.6	5.4[a]	0.5	0.549
60-74	8.0[a]	0.6	7.9[a]	0.6	6.8[a]	0.6	5.4[a]	0.5	0.265
≥75	7.5[a]	0.7	8.9[a]	0.8	5.6[a]	0.5	5.5[a]	0.5	0.943
Processed Meat									
15-18	8.4[a]	0.4	6.3[a]	0.4	7.3[a]	0.4	3.9[a]	0.2	<0.001
19-59	9.9[b]	0.5	7.5[a]	0.4	9.2[b]	0.5	4.8[a]	0.3	<0.001
60-74	9.4[a]	0.5	8.2[b]	0.4	8.1[a]	0.5	5.1[b]	0.3	<0.001

≥75	9.7[a]	0.5	7.7[a]	0.5	7.3[a]	0.4	4.5[a]	0.3	<0.001
FISH and SHELLFISH									
15-18	3.6[a]	0.4	4.4[a]	0.4	3.1[a]	0.3	2.8[a]	0.3	0.956
19-59	5.9[a]	0.5	6.2[b]	0.5	5.6[b]	0.5	4.0[b]	0.3	0.209
60-74	7.9[b]	0.6	6.7[b]	0.6	6.4[b]	0.5	4.4[b]	0.4	0.008
≥75	6.8[a]	0.6	5.9[a]	0.6	5.1[b]	0.5	3.6[a]	0.4	0.098
EGGS and EGG PRODUCTS									
15-18	1.6[a]	0.2	1.9[a]	0.2	1.2[a]	0.1	1.2[a]	0.1	0.778
19-59	1.4[b]	0.2	2.1[a]	0.2	1.2[a]	0.1	1.3[a]	0.1	0.385
60-74	1.7[a]	0.2	2.1[a]	0.2	1.3[a]	0.1	1.2[a]	0.1	0.664
≥75	1.9[a]	0.2	1.4[b]	0.2	1.4[a]	0.2	0.750[b]	0.101	0.009

SEM; standard error of the mean

[a,b,c] Mean values within a column with unlike superscript letters were significantly different (*t*-test, ANOVA with Bonferroni correction and Mann-Whitney U-test).

[*] Weighted mean of animal protein intake and its percentage

[†] *P* value for mean differences between males and females of animal protein intake (Student's *t*-test and Mann-Whitney *U*-test)

Table 4.1.4. Mean total plant protein intakes and intakes from main sources stratified by age (years) and sex

(Mean values with their standard errors, n=3083)

Plant protein food sources	Contribution to total protein intake (%)[*]				Mean plant protein intake (g/d)[*]				P[†]
	Males		Females		Males		Females		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
TOTAL PLANT PROTEIN									
15-18	29^{a}	0.7	30^{a}	0.5	23^{a}	0.5	18^{a}	0.4	<0.001
19-59	28^{a}	0.5	28^{b}	0.5	25^{a}	0.5	18^{a}	0.4	<0.001
60-74	27^{a}	0.5	29^{b}	0.5	22^{b}	0.4	17^{b}	0.3	<0.001
≥75	28^{a}	0.5	26.^{c}	0.4	20^{c}	0.4	15^{c}	0.3	<0.001
POTATOES and OTHER TUBERS									
15-18	2.8^{a}	0.2	3.1^{a}	0.2	2.9^{a}	0.1	1.8^{a}	0.1	<0.001
19-59	3.4^{b}	0.2	2.8^{a}	0.1	2.8^{a}	0.1	1.7^{a}	0.1	<0.001
60-74	4.4^{c}	0.1	4.1^{c}	0.2	3.5^{b}	0.1	2.4^{b}	0.1	<0.001
≥75	4.8^{c}	0.2	4.4^{c}	0.2	3.5^{b}	0.1	2.5^{b}	0.1	<0.001
VEGETABLES									
15-18	2.1^{a}	0.1	2.5^{a}	0.1	1.7^{a}	0.1	1.5^{a}	0.1	0.153
19-59	2.5^{a}	0.1	3.3^{b}	0.1	2.1^{b}	0.1	2.1^{b}	0.1	0.899
60-74	2.9^{b}	0.1	3.7^{c}	0.2	2.4^{c}	0.1	2.3^{b}	0.1	0.121
≥75	2.9^{b}	0.1	3.2^{b}	0.1	2.1^{c}	0.1	1.9^{c}	0.1	0.235
LEGUMES (excluded soy products)									
15-18	0.116^{a}	0.038	0.091^{a}	0.03	0.097^{a}	0.031	0.060^{a}	0.019	0.678
19-59	0.282^{a}	0.090	0.253^{a}	0.079	0.255^{a}	0.073	0.149^{a}	0.044	0.325
60-74	0.253^{a}	0.064	0.170^{a}	0.057	0.212^{a}	0.052	0.095^{a}	0.032	0.129
≥75	0.207^{a}	0.061	0.149^{a}	0.052	0.144^{a}	0.044	0.091^{a}	0.033	0.572
SOY PRODUCTS									
15-18	0.125^{a}	0.054	0.416^{a}	0.149	0.117^{a}	0.051	0.265^{a}	0.096	0.136

19-59	0.249^{a}	0.080	0.387^{a}	0.092	0.223^{a}	0.069	0.254^{a}	0.063	0.098
60-74	0.210^{a}	0.065	0.262^{a}	0.071	0.167^{a}	0.052	0.148^{a}	0.042	0.717
≥75	0.167^{b}	0.067	0.104^{b}	0.050	0.111^{b}	0.044	0.085^{b}	0.047	0.646
FRUITS									
15-18	0.828^{a}	0.09	2.1^{a}	0.2	0.690^{a}	0.079	1.2^{a}	0.1	<0.001
19-59	1.4^{b}	0.1	1.8^{a}	0.1	1.3^{b}	0.1	1.2^{b}	0.1	0.030
60-74	1.6^{c}	0.1	2.2^{b}	0.1	1.3^{c}	0.1	1.4^{c}	0.1	0.005
≥75	1.6^{c}	0.1	2.0^{b}	0.1	1.1^{c}	0.1	1.2^{c}	0.1	0.027
Fresh fruits									
15-18	0.533^{a}	0.054	1.2^{a}	0.1	0.421^{a}	0.038	0.713^{a}	0.046	<0.001
19-59	0.899^{b}	0.070	1.3^{a}	0.1	0.753^{b}	0.054	0.822^{b}	0.041	<0.001
60-74	1.2^{c}	0.1	1.9^{b}	0.1	0.927^{c}	0.051	1.2^{c}	0.1	0.001
≥75	1.4^{c}	0.1	1.9^{b}	0.1	0.993^{c}	0.062	1.1^{c}	0.1	0.010
Nuts and seeds									
15-18	0.295^{a}	0.712	0.879^{a}	0.198	0.269^{a}	0.067	0.493^{a}	0.106	0.241
19-59	0.556^{b}	0.108	0.527^{a}	0.112	0.522^{b}	0.103	0.366^{a}	0.086	0.135
60-74	0.453^{a}	0.119	0.271^{a}	0.062	0.391^{a}	0.104	0.190^{a}	0.047	0.931
≥75	0.180^{c}	0.072	0.172^{b}	0.084	0.149^{c}	0.044	0.114^{b}	0.058	0.904
CEREALS AND CEREAL PRODUCTS									
15-18	22^{a}	0.5	22^{a}	0.5	18.0^{a}	0.5	13.2^{a}	0.3	<0.001
19-59	20^{b}	0.5	19.9^{b}	0.4	18.0^{a}	0.5	12.5^{a}	0.3	<0.001
60-74	18.0^{c}	0.4	17.2^{c}	0.5	14.4^{b}	0.4	10.3^{b}	0.3	<0.001
≥75	18.4^{c}	0.4	16.1^{c}	0.4	13.1^{c}	0.3	9.1^{c}	0.2	<0.001

SEM, standard error of the mean

[a,b,c] Mean values within a column with unlike superscript letters were significantly different (*t*-test, ANOVA with Bonferroni correction and Mann-Whitney *U*-test).

[*] Weighted mean of plant protein intake and its percentage.

[†] *P* value for mean differences between males and females of plant protein intake (Student's *t*-test and Mann-Whitney *U*-test).

4.3. Associations between BMI and animal and plant protein intakes

The X^2 test showed significant positive linear relationships between BMI and animal protein intake in the elderly group (60–74 years) for both sexes (Table 4.1.5). On the other hand, negative associations were observed between BMI and plant protein intakes in the age group of adolescents (15–18 years) and adults (19–59 years) in males and females. Moreover, animal protein intake was not significantly associated with BMI of females and was not retained in the final model.

4.4. Associations between WC and animal and plant protein intakes

Table 4.1.6 shows the associations between WC and animal and plant protein intakes, which were in line with the observations for BMI. The intake of plant proteins was inversely associated with WC in all sex–age groups, except for males aged 60–74 years. Animal protein intake was positively associated with males' WC but not with females' WC.

Table 4.1.5. Generalized linear model for the associations between BMI and animal and plant protein intakes in the sex–age-specific strata

(B Coefficients with their standard errors and 95% confidence interval, n=3054)

BMI (kg/m2) †‡	**Coefficients**		**95% Confidence Interval**			
	B	**SE**	**Lower Bound**	**Upper Bound**	**Wald** χ^2	***P***
Males (n= 1535)						
Intercept	26	0.323	25	26	6420	<0.001
Animal protein	0.013	0.004	0.005	0.021	11	0.001
Plant protein	-0.036	0.009	-0.054	-0.018	15.7	<0.001
Age (years) *						
15-18	-4.3	0.261	-4.8	-3.8	277	<0.001
19-59	-0.448	0.261	-0.960	0.064	2.9	0.087
60-74	1.1	0.255	0.640	1.6	19.9	<0.001
Females (n= 1519)						
Intercept	26	0.347	25	26	5648	<0.001
Plant protein	-0.046	0.014	-0.073	-0.018	10.6	0.001
Age (years) *						
15-18	-3.9	0.319	-4.6	-3.3	152	<0.001
19-59	-1.5	0.307	-2.1	-0.899	24	<0.001
60-74	1.1	0.310	0.474	1.7	12.1	<0.001

SE, standard error of B coefficient

* Age (≥75 years) reference category

† *Male*,
Non-significant interaction: Animal protein *15-18, B=-0.015, *P*=0.250; Animal protein *19-59, B=0.001, *P*=0.947; Animal protein *60-74, B=-0.002, *P*=0.868; Plant protein *19-59, B=-0.043, *P*=0.112;
Female,

Animal protein : B=0.007, *P*=0.635

Non-significant interaction: Animal protein *15-18, B=-0.009, *P*=0.650; Animal protein *19-59, B=0.007, *P*=0.728; Animal protein *60-74, B=-0.006, *P*=0.772; Plant protein *15-18, B=-0.004, *P*=0.932; Plant protein *19-59, B=-0.007, *P*=0.871; Plant protein *60-74, B=0.032, *P*=0.516.

‡Significant interaction: *Male*, Plant protein *15-18, B=-0.061, *P*=0.027; Plant protein *60-74, B=-0.061, *P*=0.032.

Table 4.1.6. Generalized linear model for the associations between waist circumference and animal and plant protein intakes in the sex–age-specific strata

(B Coefficients with their standard errors and 95% confidence intervals, n=2874)

Waist circumference (cm) †	Coefficients		95% Confidence Interval		Wald X^2	*P*
	B	SE	Lower Bound	Upper Bound		
Males (n= 1450)						
Intercept	102	1.0	100	104	9588	<0.001
Animal protein	0.041	0.013	0.015	0.066	9.8	0.002
Plant protein	-0.137	0.03	-0.195	-0.079	21	<0.001
Age (years) *						
15-18	-20	0.839	-22	-18.4	572	<0.001
19-59	-7.9	0.845	-9.6	-6.2	88	<0.001
60-74	0.397	0.826	-1.2	2.0	0.231	0.631
Females (n= 1424)						
Intercept	96	1.1	94	99	8145	<0.001
Plant protein	-0.096	0.043	-0.180	-0.013	5.1	0.024
Age (years) *						
15-18	-18.6	0.972	-20	-16.7	365	<0.001
19-59	-11.8	0.95	-13.6	-9.9	154	<0.001
60-74	-2.3	0.951	-4.2	-0.478	6.1	0.014

SE, standard error of B coefficient

*Age (≥75 years) reference category.

† *Male*

Non-significant interaction: Animal protein *15-18, B=-0.050, *P*=0.231; Animal protein *19-59, B=-0.017, *P*=0.671; Animal protein *60-74, B=-0.007, *P*=0.877; Plant protein *15-18, B=-0.100, *P*=0.260; Plant protein *19-59, B=-0.054, *P*=0.545; Plant protein *60-74, B=-0.116, *P*=0.211;

Female

Animal protein : B=-0.024, *P*=0.623

Non-significant interaction: Animal protein *15-18, B=0.041, *P*=0.509; Animal protein *19-59, B=0.085, *P*=0.160; Animal protein *60-74, B=0.108, *P*=0.775; Plant protein *15-18, B=-0.043, *P*=0.762; Plant protein *19-59, B=-0.038, *P*=0.786; Plant protein *60-74, B=-0.053, *P*=0.725.

5. DISCUSSION

The present findings suggest that in a representative sample of the Belgian population, the most important contributors to animal protein intakes were fresh meat, cheese and milk products. In addition, cereals and cereal products were the most important contributor to plant protein intakes. Other food groups, including soya, contributed to a very low degree to the total plant protein intakes observed.

Given the lack of information on the total protein intakes from previous Belgian national nutrition surveys, we relate the present study findings to those available in other countries including the USA, Europe, Spain and China [261;432;454;576]. Differences in study design, food consumption assessment methods and food group classifications used in the various surveys should be taken into consideration when interpreting the relationships. The total energy intakes in Belgian males and females (9.5 and 6.6 MJ/d, respectively) were slightly lower than those in the UK population (males: 9·7 MJ/d; females: 6.9 MJ/d) and were considerably lower than in the Dutch population (Third Dutch National Food Consumption Survey – 1997/98) (males: 10.8–11.0 MJ/d; females: 7.8–8.4 MJ/d [226;468]. In addition, the total protein intakes expressed as percentage of energy intake were slightly lower in the Belgian population (males: 15.0%; females: 15.7%) than in the UK population (males: 16.5%; females: 16.6%). On the other hand, Belgians had similar intakes to the Dutch population (males: 14.7–15.2%; females: 15.6–16.6%), with the exception of the adolescent age group.

We have also compared the present findings with the results of the Third NHANES (1988–91) [454] and the Spanish Catalan Nutritional Survey (2002–3) [432], which used the same dietary assessment methods. It was observed that the total energy intakes of the Belgian population were lower than those of the US population (males: 10.8 MJ/d; females: 7.3 MJ/d). Belgian males and elderly females (60–75 years), however, had higher total energy intakes than the Spanish (males: 9.0 MJ/d; females: 5.7 MJ/d).

5.1. Total protein, animal and plant protein intake

According to the present study, total protein intakes were lower in the Belgian population, especially in males, when compared with US males and females (97 and 65 g/d, respectively) [454] and with Spanish males and females (97 and 79 g/d, respectively) [432] presumably due to lower intakes of animal protein. Protein intakes expressed as percentage of energy intake among Belgian sex–age-specific groups were rather similar to US adults and the elderly population (males: 15.0–16.0%; females: 15.0–17.0 %), but lower than those observed in Spanish sex–age groups (males: 18.9 %; females: 19.4 %). The Belgian population, with the exception of participants in the ≥75 years age category, however, had higher total protein, animal protein and plant protein intakes than average intakes of the European Prospective Investigation into Cancer and Nutrition Potsdam Study participants (total protein: 70 g/d; animal protein: 44 g/d; plant protein: 24 g/d) [526].

Compared with the US survey, the Belgian population had lower protein intakes from milk, yogurt, and eggs and egg products than the US population (milk and yogurt: 11.3% in males and 13.4% in females; eggs and egg products: 4.1% in males and 4.3% in females). The present results showed that fish and shellfish, and cheese contributed more to the total protein intakes in the Belgian population than in the US population. More specifically, participants in the age groups of 60–74 years and ≥75 years consumed approximately twice as much fish-derived proteins than their US counterparts (males: 5.3 %; females: 5.6%). On the other hand, fish proteins contributed more to total protein intakes in Spain (14.7%) than in Belgium (males: 3.6–7.9%; females: 4.4–6.7 %). In contrast, meat and meat products contributed less to animal intakes in the Belgian population (males: 36–37 %; females: 30–36%) than in the Spanish (39.4 %).

It was also observed that the consumption of meat proteins from subgroups including fresh meat, poultry and processed meat was lower among the Belgian than the Spanish population; females, in particular, had lower intakes of the above-mentioned meat subgroups. For example, protein intakes from poultry were much lower among Belgians (males: 7.0–8.1%; females: 7.5–8.9%) than among Spanish (14.0 %). In addition, dairy and egg protein intakes were slightly higher among the Spanish population (12.5 and 3.1%, respectively).

Fresh fruits contributed less to the total protein intakes in the Belgian population (males: 0.533–1.4%; females: 1.2– 1.9%) than in the US population (males: 1.4%; females: 1.8%) and in the Spanish population (2.0%). Protein intakes from legumes in the Belgian population were also lower than both the US (males: 2.3%; females: 2.1%) and the Spanish population (2.1%). Plant protein intake from vegetables in the present study population (males: 2.1–2.9%; females: 2.5–3.7%) was much lower than in the US population (males: 7.7%, females: 8.7%), but higher than in the Spanish population (2.3%). On the other hand, higher amounts of plant proteins from cereals and cereal products were consumed by the Belgian population in all sex–age-specific groups in comparison with the US (males: 18.0 %; females: 18.1 %) and the Spanish populations (13.0 %).

Soya protein intakes were separately analyzed in the present study, as soybeans are rich sources of protein (35–49%) and of essential amino acids [481;499]. The analysis suggested that the consumption of the Belgian population was very low and lower than those of the Chinese Guangzhou populations (males: 0.111–0.228 and 3·6 g/d; females: 0.085–0.271 and 4.1 g/d, respectively) [576] and of other East Asian populations (2.0–9.6 g/d, soya protein: total protein ratio: 3.5–15.3 %) [334]. This finding is supported by the European Prospective Investigation into Cancer and Nutrition study, which found that soya product intakes were low across all ten participating European countries [261].

5.2. BMI and animal and plant protein intake

The present results showed that animal protein intakes were positively associated with BMI of males, whereas plant protein intakes were inversely associated with the BMI of both sexes. After adjustment for potential confounders, these associations remained statistically significant. Others have reported similar results [209;320]. Hermanussen [209] concluded that the BMI of German male and female adolescents showed significant positive associations with total protein ($r = 0.143$; $P < 0.0001$) and animal protein intakes ($r = 0.151$; $P < 0.0001$). Plant protein intakes in the study of Mahon *et al*. [320] were inversely associated with the BMI of the US OW post-menopausal women.

Other studies have, however, suggested an inverse relationship between both plant and animal protein intakes and BMI [301;562], which are supported by some separate studies on animal protein intake [54;209;561] and plant protein intake [9;14;40;355;393]. In relation to animal protein intake, some studies have reported different results [320;488]. Umesawa *et al*. [488], for instance, found no association between BMI and animal protein intake, while BMI decreased slightly when females increased their animal protein consumption. Two [445;488] similar studies have reported that plant protein based diets had no significant effect on the BMI of East Asian and Western populations.

5.3. Waist circumference and animal and plant protein intake

The WHO guidelines state that risks for metabolic complications increase in men with a WC ≥102 cm and in women with a WC ≥88 cm [551]. Although the Belgian population had WC values below these cut-offs, the results of the present study indicate that Belgian females and the elderly in particular are at higher risk of being OW and obese.

The present findings also suggest that animal protein intakes might result in an increased WC for males, and plant protein intakes decrease in both males and females. This is in line with the observations that plant-based protein diets, compared with animal-based protein diets, have an inverse impact on WC of obese subjects [14;272]. For example, the results of a randomized controlled clinical trial on OW and obese people suggested that soya protein based diets resulted in bigger reductions in participants' WC than those not consuming soya protein-based diets [14]. Other studies [208;301;356;378] have, however, reported the opposite. For instance, a randomized trial involving obese adults reported no significant difference between the effect of animal protein and plant protein on WC, with total protein intakes significantly lowering the WC ($P < 0.05$) [301]. Hence, the results of randomized trials indicate that plant protein-based diets have a more protective effect against OB than animal protein-based diets [208;217;301]. Recent studies have, however, suggested that the negative relationship between animal protein and BMI refers only to OW and obese individuals and does not affect individuals with a normal BMI [1;51;159].

The mechanisms that relate animal and plant protein intakes with BMI and WC are unclear. One proposed mechanism is that animal proteins from beef, pork and poultry provide an important amount of energy and are positively associated with cholesterol and saturated fatty acid intakes. Therefore, animal protein intake may result in an increase in BMI and the risk of OW and OB. The intake of plant proteins, conversely, is considered an important factor to control body weight and improved body composition and blood lipid profiles because of their associations with lower intakes of energy, total fat, cholesterol and saturated fatty acid, and higher polyunsaturated fatty acid: saturated fatty acid ratios [57;178;246].

5.4. Strengths and limitations of the study

This nutrition survey was representative for the Belgian population. One of the limitations of the present study is the use of self-reported body composition variables including weight and height. However, WC was measured by trained dietitians. Furthermore, the present study did not consider PA and energy expenditure, factors that could have an effect on the observed associations. Information on food intake was collected via two nonconsecutive 24-h recalls, which allows statistical adjustments for within-person variability. Yet, one of the limitations of the 24-h recall method is that it does not allow quantifying proportions of non-consumers for particular food items, *a fortiori* for infrequently consumed foods. Moreover, information on the food consumption relies on individuals' memory and might therefore be biased towards misreporting. Additionally, underestimation or overestimation of portion sizes could result in inaccurate associations between dietary intake and body composition.

6. CONCLUSION

The results of the present study suggest that meat protein contributed most to animal protein intakes, and cereals and cereal products contributed to plant protein intakes. Animal and plant protein intakes were significantly different between males and females, and intakes decreased with age in both sexes. It was also observed that the consumption of legume- and soya-derived protein was very low in Belgium. Furthermore, the results indicated that animal protein intake was positively associated with BMI and WC of males, while plant protein intake was found to be negatively associated with BMI and WC of the whole population. The present study findings indicate that the intakes of plant protein could offer a potential protective effect against OW and OB.

CHAPTER 4.2

Fiber intake among the Belgian population by gender-age and gender-education groups and its association with BMI and waist circumference

Chapter based on this manuscript:

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1. ABSTRACT

Objectives

The present study was to assess total dietary fiber intake and the main contributors to fiber intake in the Belgian population by sex–age and sex–education groups and to investigate its relationship with BMI and waist circumference.

Methods

The participants of the Belgian food consumption survey (2004) were randomly selected. Information about food intake was collected using two repeated, non-consecutive 24-h recall interviews.

Results

A total of 3083 individuals (≥15 years; 1546 men and 1537 women) completed both interviews. The main contributors to total fiber intake (17.8 g/d) were cereals and cereal products (34 %; 5.9 g/d), potatoes and other tubers (18.6 %; 3.3 g/d), fruits (14.7 %; 2.8 g/d) and vegetables (14.4 %; 2.6 g/d). Legume fiber intake was extremely low (0.672 %; 0.139 g/d). In all sex–age and sex–education groups, total fiber intake was below the recommendations of the Belgian Superior Health Council. Men (21 g/d) consumed significantly more fiber than women (17.3 g/d) ($P<0.001$). Lower educated men and higher educated women reported the highest fiber intake. A significant inverse association was found between total fiber intake and WC ($\beta=-0.118$, $P<0.001$). Fruit-derived fiber was positively associated with WC ($\beta=0.731$, $P=0.001$).

Conclusion

In summary, total fiber intake was inversely associated with waist circumference, whereas fruit-derived fiber intake was positively associated with waist circumference in the Belgian population.

2. INTRODUCTION

DF is one of the nutritional compounds of vegetable foods, defined by the Codex Committee on Nutrition and Foods for Special Dietary Uses as carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by endogenous enzymes in the small intestine [115]. DF can be classified into WSF and WIF. WSFs can delay small-bowel absorption, which may reduce cholesterol absorption, but also pancreatic enzyme activity and protein digestion [408;471]. Subsequently, the colonic fermentation of fibers results in the production of gases and short-chain fatty acids [474], which causes a longer-lasting satiety and lowers the glycemic index of foods, and, consequently, attenuates the insulin response [8;324]. Due to its anti-nutritive properties and non-digestibility, WIF can increase the bulkiness of stool and faecal mass, thereby shortening transit time [474].

A decreased DF intake in Western countries is found to be associated with a higher prevalence of chronic diseases [94;173]. Epidemiological data suggest an inverse association between the consumption of DF and chronic diseases [363] and a positive association with an overall healthier profile (lower BP, lower cholesterol levels and improved insulin sensitivity) [17]. In the past decades, the decline in DF has been hypothesized as a possible determinant for increased adiposity, metabolic disorders and CVD [315]. Several recent studies have reported that DF intake may protect against adiposity and metabolic disorders [119;128;278]. Moreover, recent cohort studies and randomized control trials have shown a significant inverse association between fiber consumption and the risk of developing OB [501] and the MetS [328]. Therefore, the intake of DFs is strongly recommended by the WHO (>25 g/d) and the BSHC (40 g/d for male adolescents (14–18 years); 30 g/d for adults and female adolescents (14–18 years)) [160;551] due to the health benefits.

These fiber recommendations have been translated into food-based dietary guidelines [539] that have been developed to help the general population in choosing a healthy diet. Vandevijvere *et al.*[497] compared the Belgian food based dietary guidelines, which are based on our Belgian dietary recommendations, with the results derived from the Belgian food consumption survey. This comparison showed that Belgian dietary habits deviate importantly from the Belgian dietary guidelines. Mainly, the intake of plant products (fruits and vegetables), which are important for our fiber intake, was very low in Belgium [497]. Vandevijvere *et al*. reported that only 38 and 47% of the Belgian population consumed vegetables and fruits, respectively, whereas 82 and 52% of the Belgian population consumed bread and cereals, and potatoes and other tubers, respectively, on a daily basis. Men consumed more bread and cereal products, potatoes and grain products, and fruits than women did, though no differences were found in vegetable intake. The consumption of bread and cereals, and potatoes and grains decreased by increasing age category. The daily consumption of vegetables and fruits, however, was highest among the subpopulation aged 60–74 years old and lowest in the adolescents aged 15–18 years. Furthermore, Huybrechts & De Henauw [228] showed that the majority of Flemish preschoolers aged 2.5–6.5 years old did not meet the recommended fiber intake of the BSHC. Matthys *et al.* [325] reported that Flemish adolescents (13–18 years) also did not reach the fiber requirements of the BSHC.

When considering the above-mentioned gaps in the consumption of plant-rich foods in our Belgian diet and knowing that the prevalence of OB increased in the past years [95;460], it would be interesting to get more insight into the intake of dietary fibers among our Belgian population and its association with OW and OB.

Therefore, the main objectives of the present study were to evaluate DF intake among the Belgian population and to explore possible differences in intake among sex–age and sex–education groups. Furthermore, in view of the health effects of fibers related to OB, the association between fiber intake and BMI and WC was assessed.

3. METHODS

3.1. Sampling design and data collection

For the purpose of the present study, data from the Belgian national food consumption survey were used [123]. This survey was performed in 2004 and followed, to a large extent, the recommendations of the EFCOSUM, defining a method to monitor food consumption in nationally representative samples of all age–sex categories [79]. The target population was defined as all persons of 15 years and older residing in Belgium and willing to participate. Institutionalized individuals, persons not able to speak one of the national languages or physically or mentally unable to be interviewed were excluded from the survey. A stratified multistage sampling design was used to select the participants from the whole country covering regions of Flanders, Brussels and Walloon. The population was stratified in four sex–age groups (15–18, 19–59, 60–74 and ≥75 years). Approximately 400 individuals were included in each sex–age stratum. Adolescents were defined as participants being 15–18 years of age.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the medical ethical committee of the Scientific Institute of Public Health in Brussels. Written or verbal informed consent was obtained from all subjects. Verbal consent was witnessed and formally recorded.

3.2. Dietary intake assessment

Two repeated, non-consecutive 24-h dietary recall interviews were used to collect the food consumption data. During the first home visit, a 24-h dietary recall was performed through a computer-assisted face-to-face interview by a well-trained dietitian. The second 24 h dietary recall was repeated 2–8 weeks later (median 3 weeks). Interviews were randomly allocated to different days of the week and over a 12-month period in order to include day-to-day and seasonal variations. During the interviews, information (quantities, brand names and recipes used) was collected on all foods and beverages consumed during the preceding day.

The validated software package EPIC-SOFT (International Agency for Research on Cancer (IARC), Lyon, France),designed to obtain a very detailed description and quantification of all foods consumed, was used to obtain standardized 24-h dietary recall interviews [451]. Additionally, a book with colored photographs of foods in different portion sizes was used to support the quantification.

In the present study, 2288 different food items (from seventeen food groups and 107 food subgroups) were consumed by the respondents. DF intake was estimated based on the American Association of Analytical Chemists method [83] used in the Belgian NUBEL [370], the Dutch NEVO [361] and the USDA food composition databases [491]. DF, as defined by the American AOAC, includes NSP and indigestible carbohydrates resistant to digestive enzymes. Energy-adjusted DF intake was also calculated using the formula total fiber intake (g/d)/total energy intake (MJ/d).

3.3. Level of education

Participants were asked to report the highest degree that they had obtained during the first interview. Four categories of education level were created: (1) lower secondary; (2) vocational, technical or art; (3) general secondary; (4) higher education (bachelor, master or above).

3.4. Anthropometric measurements

Weight (kg) and height (m) were self-reported by the respondents, while WC was measured by the trained dietitians at home after 24-h recall interviews. Pregnant women reported their weight before pregnancy. BMI was calculated as weight (kg)/height (m^2). Adult participants were classified into four BMI categories according to the WHO definition: underweight (<18.5 kg/m^2); normal-weight (18.5–24.9 kg/m^2); OW (25.0–29.9 kg/m^2); OB (≥30.0 kg/m^2) [543]. The BMI of adolescent participants was classified into the same four categories according to Cole *et al*. [102] (normal-weight, OW and OB) and Flemish cut-off values (for underweight only) [512]. The cut-off criteria for adults' WC were as follows: normal, <80 cm (women) and <94 cm (men); normal to borderline, 80–88 cm (women) and 94–102 cm (men); abdominal OB, ≥88 cm (women) and ≥102 cm (men) [290]. The cut-off criteria of WC for adolescents were based on Taylor *et al*. [475].

3.5. Statistical analysis

Descriptive and statistical analyses by the sex–age and sex–education groups were performed using SPSS for Windows version 15 (SPSS, Inc., Chicago, IL, USA). Results were considered statistically significant at an a two-tailed level of 0.05. Tests for normality and equality of the variances were performed using the Kolmogorov–Smirnov and Levene's test, respectively. To obtain a normal distribution, the dietary total fiber intakes and total fiber intake from main sources were log-transformed. Descriptive statistics include mean intakes of total fiber and food group-specific fiber intakes, BMI and WC with their standard errors. Student's *t* test, ANOVA with Bonferroni correction was used to compare means between the groups.

Associations between BMI or WC (separate dependent variables) and total fiber intake or food group-specific fiber intake (independent variables) were investigated by stepwise multiple linear regression via three models: (1) unadjusted model; (2) model adjusted for age, sex, region and education level; (3) model further adjusted for total energy intake and the interactions based on model 2. The null hypothesis posited that there is no association between fiber intake and the BMI or WC (H0: B = 0). The potential effect of confounding factors such as age, sex, region and education level was analyzed by stratification to obtain the second coefficient estimate (B in model 2). Additionally, interactions between the independent variable and all effect factors and between the effect factors were examined, resulting in the third coefficient estimate (B from model 3). The results of the model 3 will be the final statistical results. Total fiber and food group-specific fiber intakes were investigated in separate models because of collinearity. Outliers were removed according to the residual method.

4. RESULTS

4.1. Study population

In total, 3083 individuals (1546 men and 1537 women) out of 7543 contacted individuals participated in the survey and completed both 24-h dietary recalls. Among all 3083 individuals, 2961 participants reported their education level, 3055 reported their weight and height, and for 2875 subjects, WC was measured.

BMI and WC of all participants differed according to sex, age and education level (Table 4.2.1). The prevalence of OW or obese men was higher compared with women, with a mean BMI of 25 and 24 kg/m^2, respectively. Of all the participants, 42% of women and 29% of men were abdominally obese. The prevalence of OW or obese subjects, based on both BMI and WC, was the highest in the groups of 60–75 and ≥75 years and in the subjects with the lowest educational level.

4.2. Total fiber intake

The mean total fiber intake in Belgium was 17.8 (1.4–57) g/d. Men consumed significantly more fiber than women (P<0.001), with the lowest and highest intakes reported by the 15–18 and the 60–74 years of sex–age groups, respectively (Table 4.2.2). With regard to the level of education, men in the lowest education group and women with a higher education level had the highest mean total fiber intakes (21 and 16.7 g/d, respectively). The energy-adjusted DF intakes, on the other hand, were significantly higher in women compared with men (P<0.001), increased by ageing and was the highest in the lowest educated women in the sex–education groups.

The mean DF intake of the Belgian population was approximately half of the recommended intake by the BSHC (Table 4.2.2) [160], with the majority (63% men and 89% women) not meeting the recommendations. Fiber intakes of Belgian adults were too low according to several international guidelines for adults, such as the WHO (>25 g/d) [551], the USDA (men, 38 g/d; women, 25 g/d) [491], the Institute of Medicine of the National Academies (men aged 19–50 years, 38 g/d; men ≥50 years, 30 g/d; women aged 19–50 years, 25 g/d; women ≥50 years, 21 g/d) [484] and the British Nutrition Foundation (18.0 g/d NSP, approximately 24 g/d total dietary fiber) [75]. Likewise, the adolescent population (men, 17.8 g/d; women, 15.0 g/d) reported intakes below the dietary reference intakes of the Institute of Medicine of the National Academies (men, 38 g/d; women, 26 g/d) [484] and Williams' Age plus 5 guideline (19.0–23 g/d) [535].

Table 4.2.1. Characteristics of the participants of the Belgian national food consumption survey (2004-5)

(Mean values with their standard errors)

	Population	BMI*						Waist circumference†				
		(kg/m²)		Prevalence (%)				(cm)		Prevalence (%)		
Characteristics	***n***	**Mean**	**SEM**	**Under-weight**	**Normal**	**Over-weight**	**Obesity**	**Mean**	**SEM**	**Normal**	**Borderline**	**Abdominal obesity**
Gender	3083											
Men	1546	25	0.1	3.1	52	34	10.1	88	0.7	43	27	29
Women	1537	24	0.1	5.7	58	25	10.5	80	0.7	39	19	42
Age (years)	3083											
15-18	762	21	0.1	9.7	79	10.2	1.3	76	0.6	72	20	7.6
19-59	828	24	0.2	3.7	60	26	10.1	81	1	51	24	25
60-75	789	26	0.2	1.0	36	44	18.6	91	0.9	20	27	53
≥75	704	25	0.2	3.2	46	40	10.8	90	1.2	19	22	59
Education	2961											
Lower secondary or less	967	26	0.1	2.2	41	40	16.5	97	0.4	21	23	56
Vocational, technical or art	734	24	0.2	5.4	60	27	7.6	87	0.5	49	26	25
General secondary	530	22	0.2	6.0	70	20	4.2	83	0.6	57	22	21
Higher	730	24	0.2	4.7	60	27	8.6	89	0.5	47	23	30
Region												
Flanders	955	24	0.1	3.7	58	29	9.5	84	0.6	41	24	35
Brussels	233	24	0.3	6.5	58	27	7.8	83	1.5	43	28	29

Walloon	918	25	0.1	5.1	50	33	12.5	86	0.8	40	21	38

SEM, standard error of the mean

*BMI categories adults: underweight: < 18.5 kg/m², normal-weight: 18.5-24.9 kg/m², overweight: 25.0-29.9 kg/m², obese: ≥30.0 kg/m² [543]. For adolescents, the categories were assigned according to the cut-off values for children and adolescents developed by Cole et al.[102] and according to the Flanders growth charts [512].

†Waist circumference categories for adults: normal, <94 cm (men) and < 80 cm (women); borderline: 94-102 cm (men) and 80-88 cm (women); obesity: ≥102 cm (men) and ≥88 cm (women) [290]. For adolescents, the categories were assigned according to the cut-off values given by Taylor et al. [475].

Table 4.2.2. Reported and recommended daily total dietary fiber intakes and the percentage of the subjects in agreement with the recommendations of the Belgian Health Council [160], stratified in gender-age and gender-education groups

(Mean values with their standard errors)

	Men					Women*					Subjects (%)†	
	Reported (g/d)		Energy-adjusted (g/(MJ*d))		Recommended (g/d)	Reported (g/d)		Energy-adjusted (g/(MJ*d))		Recommended (g/d)		
Stratification	Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM		Men	Women
Age (years)												
15-18	17.8[a]	0.4	1.7[a]	0.1	40	15.0[a]	0.3	2.1[a]	0.1	30	0	0.8
19-59	19.5[a]	0.4	2.0[b]	0.1	30	15.8[a]	0.3	2.4[b]	0.1	30	12.4	2.1
60-75	21[b]	0.4	2.4[c]	0.1	30	17.5[b]	0.4	2.8[c]	0.1	30	14.3	5.6
≥75	19.8[a]	0.4	2.5[c]	0.1	30	16.2[a]	0.4	2.7[c]	0.1	30	10.2	2.7
Education												
Lower secondary or less	20.6[a]	0.4	2.5[a]	0.1	30	16.3[a]	0.3	2.6[a]	0.1	30	14.6	3.5
Vocational, technical or art education	19.1[b]	0.4	2.0[b]	0.1	30	15.1[a]	0.3	2.3[b]	0.1	30	9.6	0.9
General secondary education	18.8[a]	0.5	2.0[b]	0.1	30	16.5[b]	0.4	2.3[b]	0.1	30	6.3	3.2
Higher education	19.1[a]	0.4	2.1[b]	0.1	30	16.7[b]	0.3	2.5[a]	0.1	30	1.1	3.0

SEM, standard error of the mean

[a,b,c] Mean values within a column with unlike superscript letters were significantly different (P<0.05, ANOVA with Bonferroni correction after log transformation).

[*] Mean value was significantly different between men and women (P<0.05; Student's t-test after log transformation).

[†] Percentage of participants meeting the recommended dietary fiber intake of the Belgian Health Council.

4.3. Main food groups contributing to fiber intake

Five food groups contributed to 82% of the total fiber intake in Belgium (corresponding with a mean intake of 14.7 g/d). In all sex–age (Table 4.2.3) and sex–education (Table 4.2.4) groups, cereals and cereal products contributed most (34 %; 5.9 g/d), with bread, crispbread and rusks as the main sources within this category, followed by potatoes and other tubers (18.6 %, 3.3 g/d), fruits (14.7 %, 2.8 g/d) and vegetables (14.4 %, 2.6 g/d). Interestingly, the subgroup 'bread, crispbread and rusks' of cereals and cereal products contributed significantly less in the adolescent group compared with the older groups (P<0.05), whereas 'other cereal products' from the subgroups of "salty biscuits and aperitif biscuits", "dough and pastry, pasta and rice" and "breakfast cereals", in contrast, contributed most to adolescents' intake. The intake of legume-derived fibers was extremely low (0.672 %; 0.139 g/d) in all sex–age and sex–education groups. The contributions of fruits and vegetables were the largest in the elderly population (60–74 years in particular) and the lowest in adolescents. In general, potato-derived fiber intakes decreased with the level of education. The contribution of other cereal products was the lowest for lower secondary educated participants, whereas higher educated subjects consumed more fibers from vegetables and fruits.

4.4. Anthropometric indices and total and specific fiber intake

Associations between BMI or WC, on the one hand, and total or food group-specific fiber intake, on the other hand, were investigated by stepwise multiple linear regression (Table 4.2.5). In model 1, crude BMI was associated with fiber intake from potatoes and other tubers, vegetables, fruits, and cereals and cereal products. After adjustment for age, sex, region and education level (model 2), a significant association was observed between BMI and the intake of fibers from cereals and cereal products (B = -0.045, P=0.025), but no association was found after adjustment for energy intakes and interactions with and between the effect factors (model 3). According to model 3, WC, on the other hand, was inversely related to the total fiber intakes (B = -0.118, P<0.001) and positively related to fruit-derived fiber intakes (B = 0.731,P=0.001).

Table 4.2.3. Contribution (g/d) of different food groups to the total fiber intake among the study population, stratified by gender-age groups

(Mean values with their standard errors)

Food sources	Age (years)							
	15-18		19-59		60-75		≥75	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
MEN								
TOTAL INTAKE OF MAIN SOURCES	14.2[a]	0.3	16.4[b]	0.4	17.6[c]	0.4	16.7[b]	0.4
POTATOES and OTHER TUBERS	3.5[a]	0.1	3.5[a]	0.2	4.4[b]	0.1	4.4[b]	0.2
VEGETABLES	2.1[a]	0.1	2.6[b]	0.1	3.1[c]	0.1	2.6[c]	0.1
LEGUMES	0.097[a]	0.029	0.255[a]	0.073	0.220[a]	0.060	0.137[a]	0.044
FRUITS								
All fruit	1.5[a]	0.1	2.5[b]	0.2	3.3[c]	0.2	3.2[c]	0.2
Fresh fruit	1.4[a]	0.1	2.2[b]	0.1	3.1[c]	0.2	3.1[c]	0.2
Nuts and seeds	0.115[a]	0.030	0.243[b]	0.051	0.207[a]	0.057	0.083[a]	0.040
CEREALS and CEREAL PRODUCTS								
All	7.0[a]	0.2	7.6[a]	0.3	6.7[a]	0.2	6.4[b]	0.2
Bread, crispbread and rusks	5.1[a]	0.2	6.4[b]	0.3	6.1[b]	0.2	5.9[b]	0.2
Other cereal products	1.9[a]	0.1	1.2[b]	0.1	0.557[c]	0.051	0.483[c]	0.063
WOMEN								
TOTAL INTAKE OF MAIN SOURCES*	11.7[a]	0.3	12.9[b]	0.3	14.7[c]	0.3	13.4[b]	0.3
POTATOES and OTHER TUBERS*	2.2[a]	0.1	2.1[a]	0.1	3.0[b]	0.1	3.2[b]	0.1
VEGETABLES	1.9[a]	0.1	2.7[b]	0.1	2.9[b]	0.1	2.5[a]	0.1
LEGUMES	0.060[a]	0.019	0.141[a]	0.043	0.102[a]	0.038	0.097[a]	0.037
FRUITS								
All fruit*	2.4[a]	0.1	2.7[b]	0.1	3.7[c]	0.2	3.3[c]	0.2
Fresh fruit*	2.2[a]	0.1	2.5[b]	0.1	3.6[c]	0.2	3.3[c]	0.2
Nuts and seeds	0.191[a]	0.042	0.141[a]	0.03	0.094[a]	0.023	0.065[b]	0.037
CEREALS and CEREAL PRODUCTS								
All*	5.1[a]	0.1	5.3	0.2	5	0.2	4.2[b]	0.1
Bread, crispbread and rusks*	3.7[a]	0.1	4.3[b]	0.2	4.5[b]	0.1	4.0[b]	0.1
Other cereal products	1.5*[,a]	0.1	1.0[b]	0.1	0.466[c]	0.053	0.224[d]	0.029

SEM, standard error of the mean

[a,b,c,d] Mean values within a row with unlike superscript letters were significantly different (*P*<0.05, ANOVA with Bonferroni correction after log transformation)

* Mean values were significantly different between men and women (*P*<0.05, Student's *t* test after log transformation)

Table 4.2.4. Contribution (g/d) of different food groups to the total fiber intake among the study population, stratified in gender-education groups

(Mean values with their standard errors)

Food sources	Education							
	Lower secondary or less		Vocational, technical or art education		General secondary education		Higher education	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
MEN								
TOTAL INTAKE OF MAIN SOURCES	17.6[a]	0.4	15.8[b]	0.4	15.3[b]	0.4	15.9[b]	0.4
POTATOES and OTHER TUBERS	4.7[a]	0.1	4.2[b]	0.2	3.2[c]	0.2	3.3[c]	0.1
VEGETABLES	2.7[a]	0.1	2.4[b]	0.1	2.3[b]	0.1	2.8[a]	0.1
LEGUMES	0.240[a]	0.069	0.149[a]	0.044	0.127[a]	0.042	0.185[a]	0.049
FRUITS								
All fruit	2.9[a]	0.2	2.1[b]	0.1	2.3[b]	0.2	3.0[a]	0.2
Fresh fruit	2.8[a]	0.2	1.9[b]	0.1	2.2[b]	0.2	2.8[a]	0.2
Nuts and seeds	0.118[a]	0.036	0.132[a]	0.034	0.140[a]	0.038	0.236[b]	0.060
CEREALS and CEREAL PRODUCTS								
All	7.0[a]	0.2	7.1[a]	0.2	7.3[a]	0.3	6.6[a]	0.2
Bread, crispbread and rusks	6.4[a]	0.2	6.0[a]	0.2	5.6[b]	0.2	5.4[b]	0.2
Other cereal products	0.560[a]	0.056	1.1[b]	0.1	1.7[c]	0.1	1.1[b]	0.1
WOMEN								
TOTAL INTAKE OF MAIN SOURCES*	13.5[a]	0.3	12.2[b]	0.3	13.2[a]	0.4	13.6[a]	0.3
POTATOES and OTHER TUBERS*	3.2 [a]	0.1	2.5[b]	0.1	2.5[b]	0.1	2.1[c]	0.1
VEGETABLES	2.5[a]	0.1	2.2[b]	0.1	2.5[a]	0.1	3.0[c]	0.1
LEGUMES	0.135[a]	0.038	0.108[a]	0.049	0.100[a]	0.031	0.036[a]	0.019
FRUITS								
All fruit*	2.9[a]	0.1	2.8[a]	0.2	3.2[a]	0.2	3.2[a]	0.2
Fresh fruit*	2.9[a]	0.1	2.6[b]	0.2	3.0[a]	0.2	3.0[a]	0.2
Nuts and seeds	0.040[a]	0.016	0.150[a]	0.037	0.191[b]	0.046	0.177[a]	0.044
CEREALS and CEREAL PRODUCTS								
All	4.8[a]	0.1	4.7[a]	0.2	5.0[a]	0.2	5.3[b]	0.2
Bread, crispbread and rusks*	4.4 [a]	0.1	3.7[b]	0.1	3.9[b]	0.2	4.3[a]	0.2
Other cereal products*	0.403[a]	0.104	1.0[b]	0.1	1.1[b]	0.1	0.925[b]	0.065

SEM, standard error of the mean

[a,b,c] Mean values within a row with unlike superscript letters were significantly different (*P*<0.05;ANOVA with Bonferroni correction after log transformation)

* Mean values were significantly different between men and women (*P*<0.05; Student's t test after log transformation)

Table 4.2.5. Stepwise multiple linear regression analysis of the potential associations between BMI and waist circumference, and total and food group-specific fiber intakes among participants of the Belgian national food consumption survey (2004-2005)

(Coefficients with their standard errors and 95% confidence intervals)

	B **Coefficient**	**SE**	**95 % CI**		***P* value**
	Dependent variable: BMI (*n*=3055)*†‡				
Food groups§					
Potatoes and other tubers					
Model 1	0.139	0.029	0.081	0.197	<0.001
Model 2	0.022	0.023	-0.024	0.067	0.353
Model 3	-0.009	0.028	-0.064	0.046	0.754
Vegetables					
Model 1	0.112	0.037	0.039	0.186	0.003
Model 2	0.065	0.034	-0.002	0.131	0.057
Model 3	0.056	0.034	-0.01	0.123	0.095
Fruits					
Model 1	0.107	0.025	0.058	0.157	<0.001
Model 2	0.022	0.023	-0.024	0.067	0.353
Model 3	0.024	0.023	-0.021	0.069	0.297
Cereals and cereal products					
Model 1	-0.077	0.020	-0.118	-0.037	<0.001
Model 2	-0.045	0.020	-0.084	-0.006	0.025
Model 3	-0.019	0.022	-0.063	0.025	0.391
	Dependent variable: Waist circumference (*n*=2875)*† ‡				
Total fiber‖					
Model 1	0.172	0.037	0.099	0.244	<0.001
Model 2	-0.091	0.03	-0.150	-0.032	0.003
Model 3	-0.118	0.032	-0.181	-0.055	<0.001
Food groups¶					
Potatoes and other tubers					
Model 1	0.876	0.102	0.676	1.076	<0.001
Model 2	-0.003	0.086	-0.171	0.165	0.972
Model 3	-0.028	0.085	-0.195	0.14	0.744
Vegetables					
Model 1	0.288	0.131	0.031	0.545	0.028
Model 2	0.063	0.105	-0.143	0.269	0.548
Model 3	0.099	0.104	-0.105	0.303	0.342

Fruits					
Model 1	0.334	0.087	0.164	0.505	<0.001
Model 2	-0.066	0.07	-0.204	0.072	0.349
Model 3	0.731	0.212	0.316	1.146	0.001

SE, standard error of coefficient B

* Model 1, unadjusted; model 2, adjusted for age, sex, region and level of education; model 3, model 2 further adjusted for total energy intake and interactions with and between the effect factors.

† Legume-derived fiber intake was not retained in the models for both BMI and WC, whereas total fiber intakes and cereal-derived fiber intakes were not retained in the model for BMI and WC, respectively.

‡Women, lower secondary education and Walloon region are as reference in the models.

§ Model 2. Significantly positively associated variable: age; significantly negatively associated variables: sex, general secondary education, higher education, Flanders region and Brussels region. Model 3. Significantly positively associated variables: age, sex, higher education and Brussels region; significantly negatively associated variables: general secondary education and Flanders region. Significantly positively associated interactions: vocational, technical or art * sex, higher education * age and Brussels region x age; significantly negatively associated interactions: sex * age, vocational, technical or art * age, general secondary education * sex, general secondary education * age, higher education * sex, and cereal fibre * vocational, technical or art.

|| Model 2. Significantly positively associated variable: age; significantly negatively associated variables: sex, general secondary education and Brussels region. Model 3. Significantly positively associated variables: age and Brussels region; significantly negatively associated variables: sex and general secondary education. Significantly positively associated interactions: general secondary education * age and total fibre * higher education; significantly negatively associated interactions: sex * age, higher education * sex and Brussels region * age

¶ Model 2. Significantly positively associated variable: age; significantly negatively associated variables: sex, general secondary education and higher education. Model 3. Significantly positively associated variables: age and higher education; significantly negatively associated variables: sex and general secondary education. Significantly positively associated interaction: general secondary education *age; significantly negatively associated interactions: higher education * age, Brussels region * age, fruit fiber * sex, cereal fiber* vocational, technical or art, and cereal fiber * general secondary education.

5. DISCUSSION

5.1. Total and food group-specific fiber intake

The total daily fiber intakes in this large population-based national nutrition survey were on average 17.8 g and below (men 63 %, women 89 %) the recommendations proposed by the BSHC [160].

Compared with the study reported by Matthys *et al*. [325] involving Flemish adolescents (13–17 years) living in Ghent and using 7d food records, the male adolescents participating in the present study consumed considerably less DF than Ghent adolescents (mean 21 g/d), whereas the female adolescents from Ghent had a slightly higher average DF intake (mean 15.9 g/d). After adjustment for energy intake, however, male adolescents from the Belgian food consumption survey had slightly lower intakes than reported in the study of Matthys *et al*. (1.8 g/MJ * d), while female adolescents had higher intakes than those in the study of Matthys *et al*. (1.9 g/10 MJ).

The DF intakes of the Belgian population were comparable to the European levels (male adolescents, 14.0–26 g/d; female adolescents, 14.0–22 g/d; male adults, 18.0–26 g/d; female adults, 16.0–26 g/d; elderly men, 15.0–31 g/d, and elderly women, 16.0–23 g/d) [150]. Belgian men had similar DF intakes as Catalan men in Spain using the same method in 10- to 75-year-old subjects, while Belgian women in all age groups consumed substantially less DFs than Spanish women (Spanish 17.0 g/d) [432]. Additionally, Spanish male adolescents (mean 18.7 g/d) had slightly higher DF intakes than Belgian male adolescents (17.8 g/d), whereas Belgian adolescents had slightly higher DF intakes than Italian adolescents via 24 h dietary records (Belgium: men, 17.8 g/d; women, 15.0 g/d; Italy: men, 17.0 g/d; women, 14.0 g/d) [291].

According to two American national nutrition surveys using 24-h recalls (1988–91: all age categories; 1988–2004: adults), lower total fiber intakes were reported among the US population (14.0–16.7 and 16.0–19.0 g/d, respectively) [6;379]. Furthermore, based on a Japanese dietary survey among the general population, Japanese and Belgian men had similar fiber intakes, whereas Japanese women (14.7–21 g/d) consumed comparatively more fibers than Belgian women in all age groups [173]. However, Japanese men < 30 years (13.7 g/d) consumed a substantially lower amount of fibers compared with the same Belgian population group (18.1 g/d).

In the present study, the most important contributors to DF intake were cereals and cereal products (bread, crispbread and rusks in particular), followed by potatoes and other tubers, vegetables and fruits (mainly fresh fruits). Similar food groups were identified as the major contributors in Italian adolescents [291]. Results of the Spanish and Japanese national surveys, on the other hand, indicate that potato-derived fiber intakes contributed much more to the Belgian (14.4 %, 3.3 g/d) than to Spanish and Japanese total fiber intakes (6.0 %, approximately 1.1 g/d and approximately 1.5 g/d, respectively) [173;432]. While the contribution of cereal-derived fibers was higher for Belgians than for Spanish persons (34 %, 5.9 g/d *v.* 28 %, approximately 5·2 g/d), the contribution of fruit-derived fibers was considerably lower in Belgians compared with Spanish (14.7 %, 2.8 g/d *v.* 22 %, approximately 3.9 g/d). Additionally, the contribution of fruit-derived fibers in the Belgian was similar to that in the Japanese population (13.5 %, 2.5 g/d). The fiber intakes from the remaining food groups, including legumes, nuts and seeds, and vegetables, were lower in this Belgium sample than in the other surveys. In particular, the consumption and contribution of legume-derived fibers were extremely low compared with Spanish (12.1 %, approximately 2.2 g/d) [432] and Japanese (14.1 %, approximately 2.6 g/d) [173] reports.

To the best of our knowledge, no published Belgian data are available describing DF intakes stratified by level of education. There are indications that a higher socio-economic status such as education, family income and occupational level is associated with higher intakes of DFs [60;214;226;285;578]. Nevertheless, in our Belgian study, lower secondary educated men and higher educated women reported the highest total fiber intakes. In all sex–education groups, cereals and cereal products contributed most to the total fiber intake. The contribution of cereal products other than bread, crispbread or rusks was the lowest for lower secondary educated men and women, whereas higher educated persons consumed more fibers from vegetables and fruits. In general, potato-derived fiber intakes decreased with the level of education. Moreover, some recent studies have observed that fiber intake through the consumption of vegetables, fruits and cereals increases with participants' socio-economic status and education level [60;285].

5.2. Associations between dietary fiber intake and BMI and waist circumference

Although BMI is not an accurate indicator of body composition, it is a well-known predictor of OB in the general population [319]. WC is a better and stronger predictor of abdominal OB and OB-related health risks [92]. Hence, in the present study, both BMI and WC were used in order to find more precise associations between DF intake and body composition.

There are indications that the consumption of DFs may have beneficial effects, such as lowering body weight, BMI and WC [4;86;148;219;302;329;348;463;479;486;502]. In the present study, total DF intakes were significantly inversely associated with WC, but not with BMI. In line with the present findings, Ventura *et al.* [502] reported that higher total DF intakes correlated with lower WC. Moreover, Liese *et al.* [302] found that total fiber intakes were inversely associated with both BMI (B= -0.795, P=0.013) and WC (B=-1.9, P=0.008) after adjustment for age, sex and socio-economic status. Du *et al.* [140] observed that dietary fiber intakes were inversely associated with body-weight gain and WC increases among Europeans. Conversely, one cross-sectional study involving adults aged 60–80 years and one randomized controlled trial involving breast cancer patients aged 18–70 years reported no significant effects of DFs on BMI or body weight [329;479]. Additionally, in one recent cross-sectional study involving 5783 Chinese adults aged 20–59 years, higher total DF intakes correlated with increased BMI [463].

Although cereal-derived fiber intake was the most important contributor in all sex–age and sex–education groups in the present study, it was not significantly associated with BMI nor WC. A European prospective cohort study [140], however, suggested that fibers derived from cereals, more than those from vegetables or fruits, may have a role in the prevention of body-weight and WC gain. In addition, recent studies have found that cereal derived fiber intakes were inversely associated with BMI and WC [189;328;329;363]. A recent systematic review has suggested that the consumption of cereal fiber led to more health benefits in the prevention of T2D by improving insulin sensitivity and increased bowel movements without adverse effects [402]. Furthermore, one prospective cohort study and one cross-over study have stated that a high cereal fiber intake with a low glycemic load was inversely associated with the risk of T2D compared with lower cereal fiber intake and higher glycemic load [175;424]. Surprisingly, in the present study, the intake of fruit-derived fibers was positively associated with WC. Conversely, McKeown *et al*. [329] found no significant associations between the intake of fibers from vegetables or fruits and body composition. Moreover, some recent studies have described an inverse relationship between fruit- and vegetable-derived fiber intakes and body weight and BMI [271;427]. According to recent reviews, high fiber intakes through the consumption of vegetables and fruits would have no direct effect on body weight, but may exert indirectly health-promoting activities related to body composition [162;259].

In the present study, we controlled for potential modifier effects and observed that age, sex, region, education, energy intake and certain interactions affected the linear association between DF intakes and BMI and WC. Similarly, region, age, sex and level of education have been described as potential confounding factors for the effect of fiber intake on measures of OB [4]. Byrd-Williams *et al*. [86] found that DF intakes were inversely associated with the BMI of men and women (18–24 years), and with the WC of men. Howarth *et al*. [219], on the other hand, reported that DF intakes were inversely associated with the BMI of women aged 20–59 years, but not of men in the same age range.

5.3. Strengths and limitations

This national nutrition survey can be seen as the largest survey covering all the provinces and language regions in Belgium so far. The present study is the first one evaluating DF intake stratified by sex–age and sex–education, as well as assessing the association with anthropometric indices including BMI and WC in the Belgian population.

Yet, some limitations of the present study are the low response rate (42%) and the use of two 24-h recalls to assess DF intakes. Although, no doubt, willingness to participate leads to some selection bias as volunteers are generally more concerned with health and diet than others, the present study population represents a more general population of Belgium in comparison with other studies, which are mostly restricted to local areas. In addition, all seasons and days of the week were almost equally represented. Another limitation related to the sampling is the broad age range of the young adult group (19–59 years), which could lead to imprecise interpretations of the results obtained for that age group. Differences attributed to this age group may be applicable only to a smaller subset with more significant differences, and this may have implications for policy recommendations.

A limitation of 24-h dietary recalls is that it does not allow quantifying proportions of non-consumers for particular food items, *a fortiori* for infrequently consumed foods. Moreover, accuracy of collected data relies on the individual's ability to remember foods and beverages consumed in the past 24-h, and might, therefore, be biased towards misreporting. In this respect, the 24-h dietary recalls were performed through computer-assisted EPIC-SOFT and face-to-face interviews to guide the participants to report all their consumption, even the easily forgotten snacks.

Another limitation of this survey is the fact that weight and height were self-reported. However, the strength of this survey is that WC was measured by trained dietitians. When interpreting the results, it could be that this difference in recording method (self-reported *v.* measured) is partly responsible for the differences found between BMI and WC in the present study. Yet, the inclusion of both measures is an important strength of the present study, as these are not similar but complementary parameters for body composition due to their independent contribution to the prediction of total and abdominal OB [242].

Additionally, a large number of missing values of WC might be a factor leading to a biased sample, as obese people might more often refuse to have a WC measurement. Then, the underestimated WC could bias the association between WC and DF intake in the present study. However, the authors performed some additional sensitivity analyses (results not shown), which showed no significant differences in BMI or fiber intake between the group with missing WC data and those included in the WC analyses.

At last, it is noteworthy that soluble and insoluble DF intake should be analyzed separately for further knowledge, but due to the information in the food composition databases, this was not possible. Biomarkers as well need to be considered to investigate the association with DF intake and glycemic index in future studies.

5.3. Recommendations

The present study findings indicate unfavorably low fiber intakes among our Belgian population. Enhancing the daily amount of vegetables, fruits, legumes, nuts and seeds could contribute importantly to higher fiber intakes. However, in the battle against OB, one should keep in mind that the total energy intake should not be influenced by the enhancement of these food groups, which could necessitate a concomitant decrease in other (less fiber rich and energy-dense) foods such as soft drinks, candies and refined bakery products. If public health policies for the increase in fiber intakes are to be effective, policy development and implementation need to target the main sources of DF in various populations. Also manufacturers could be stimulated to reformulate existing products to incorporate more whole grains.

6. CONCLUSION

This survey provides information on the consumption of total and food group-specific fibers in Belgium. The most important contributor was cereals and cereal products. Total fiber intakes were lower than the recommended intake in all sex–age and sex–education groups. The DF intakes increased with age. Lower educated men and higher educated women consumed most DF. Although these results show that Belgians did not consume enough fiber, a significant inverse relationship was observed between total fiber and cereal-derived fiber intakes and WC.

CHAPTER 5

RESULTS FROM EUROPEAN ADOLESCENTS

CHAPTER 5.1

Dietary animal and plant protein intakes and their associations with obesity and cardio-metabolic indicators in European adolescents: the HELENA cross-sectional study

Chapter based on this manuscript:

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1. ABSTRACT

Background

Previous studies suggest that dietary protein might play a beneficial role in combating obesity and its related chronic diseases. Total, animal and plant protein intakes and their associations with anthropometry and serum biomarkers in European adolescents using one standardized methodology across European countries are not well documented.

Objectives

To evaluate total, animal and plant protein intakes in European adolescents stratified by gender and age, and to investigate their associations with cardio-metabolic indicators (anthropometry and biomarkers).

Methods

The current analysis included 1804 randomly selected adolescents participating in the HELENA study (conducted in 2006-2007) aged 12.5-17.5 years (47% males) who completed two non-consecutive computerized 24-h dietary recalls. Associations between animal and plant protein intakes, and anthropometry and serum biomarkers were examined with General linear Model multivariate analysis.

Results

Average total protein intake exceeded the recommendations of World Health Organization and European Food Safety Authority. Mean total protein intake was 96 g/d (59% derived from animal protein). Total, animal and plant protein intakes (g/d) were significantly lower in females than in males and total and plant protein intakes were lower in younger participants (12.5-14.9 years). Protein intake was significantly lower in underweight subjects and higher in obese ones; the direction of the relationship was reversed after adjustments for body weight (g/(kg.d)). The inverse association of plant protein intakes was stronger with BMI z-score and body fat percentage (BF%) compared to animal protein intakes. Additionally, BMI and BF% were positively associated with energy percentage of animal protein.

Conclusions

This sample of European adolescents appeared to have adequate total protein intake. Our findings suggest that plant protein intakes may play a role in obesity among European adolescents. Further longitudinal studies are needed to investigate the potential beneficial effects observed in this study in the prevention of obesity and related chronic diseases.

2. INTRODUCTION

The prevalence of OW and OB in adolescents, defined on the basis of body mass [102], has increased rapidly worldwide. In 2010, the estimated prevalence of OW and OB in European children and adolescents was approximately 38%, including 10% OB [310]. As a consequence of OB-related co-morbidities, over 20000 children suffer from type 2 diabetes and more than 400000 have impaired glucose levels [310]. Childhood OW and OB both influence long-term health and evidence suggest an association with coronary events and mortality later in life[37;130].

Nutrition during the early years of life is a critical factor of OB in adolescence further impacting on adulthood OW and OB, and the consequences of chronic diseases [309;478]. High protein intakes were reported to improve cardiovascular risk factors including abdominal OB, dyslipidemia, glucose intolerance, and hypertension in European children (5-18 years) [116]. Previous randomized trials [147;294] suggest that a high-protein diet defined as ≥20% of total energy lowers the risk of adolescent OW and promotes weight maintenance [458]. The association between dietary protein intake and adolescent OW and OB has mainly been investigated in relation to its increased thermic effect and satiety when compared to fats and carbohydrates [294;528]. Others, however, have reported that higher protein content in the diet did not confer any benefit in the treatment of OB among children 9-18 years old [413].

The debate on protein sources is still ongoing, addressing the nutritional quality of dietary proteins based on their amino acids composition. The protein quality or biological value of proteins from animal sources is high, whereas most plant proteins lack one or more essential amino acids and are therefore considered as incomplete proteins. What some seem to be concerned with is that the majority of high-protein foods are significant sources of fat and/or sugar as well (such as meat and meat products, cheese, and dairy desserts). Hermanussen *et al.* reported a positive correlation between the energy contribution of animal proteins to the diet and the BMI in adolescents [209]. On the other hand, Bradlee *et al.* found no association between OB and meat consumption among adolescents [72], while, plant-based diets were inversely associated with normal BMI in children in Hermanussen's study [209]. A Western dietary pattern high in animal sources is associated with an increased risk of MetS [155;494], whereas diets high in fruits, vegetables and whole grains are associated with a decreased risk [154]. Evidence showed that plant protein, soy in particular, can bind phytoestrogen compounds to stimulate lipid metabolism resulting in a better blood profile, by lowering TC, TG, LDL-C and reducing insulin resistance [62;89].

The aim of the current study was to evaluate total, animal and plant protein intakes in European adolescents and to investigate their association with cardio-metabolic indicators (anthropometry: BMI z-score and BF%; and biomarkers: TC, TG, LDL-C, VLDL-C, HDL-C, CRP, glucose, insulin and leptin).

3. METHODS

3.1. Survey population

The Healthy Lifestyle in Europe by Nutrition in Adolescence-Cross Sectional Study (HELENA-CSS) is a European Commission funded project on lifestyle and nutrition among adolescents from 10 cities of European countries: Stockholm, Athens, Heraklion, Rome, Zaragoza, Ghent, Lille, Dortmund, Vienna, and Pecs that ran between October 2006 and December 2007. Due to logistical reasons, adolescents from Heraklion and Pecs were excluded for the dietary intake assessments. A multi-stage random cluster sampling procedure was used to select 3528 adolescents, stratified by geographical location, age and socioeconomic status (SES). Schools were randomly selected after stratification to guarantee diversity of the sample in culture and SES.

Male and female adolescents, aged 12.5-17.5 years, not participating simultaneously in a clinical trial, free of any acute infection lasting less than 1 week before inclusion year, and who provided two 24-h recall interviews with valid information and complete anthropometric measurements, were included in the final analysis of the current study. Details on sampling procedures, study design and non-respondents have been reported elsewhere [50;341].

The study was approved by the Research Ethics Committees of each city involved. Written informed consent was obtained from the adolescents' parents and the adolescents themselves [48].

3.2. Dietary intake assessment

Two non-consecutive computerized 24-h dietary recalls (HELENA-DIAT), instructed by dieticians/researchers, were used to collect food consumption data. During interviews, adolescents were allowed to ask questions and following completion the recall was checked for completeness. Each participant was asked to complete the recall twice in a time-span of 2 weeks during the school time.

HELENA-DIAT is a self-administered computer program based on the YANA-C [504], consisting of a single computerized 24-h recall with a structured program based on six meal occasions. The validated YANA-C [504], was designed to obtain a detailed description and quantification of foods consumed, and eventually included more than 800 food items hierarchically organized in 25 food groups, and about 2600 colored photograph sets of more than 300 food items in different portions [503;505].

Dietary intakes were linked to the German Food Code and Nutrient DataBase (BLS (Bundeslebensmittelschlüssel), version II.3.1, 2011) [161]. However, the estimated percentage of animal and plant protein intakes were calculated by linking the 24-h recall food consumption data to the Belgian NUBEL [370], the Dutch NEVO [361] and the USDA [490] food composition databases which used the Kjeldahl method for analyzing protein [30], because no differentiation was made between plant and animal proteins in the BLS database. Protein intakes were calculated in absolute terms (g/d) and relative terms (energy percentages (E%); per kg body weight).

Under-reporters, excluded in the current study, were considered as individuals with a ratio of energy intake over estimated basal metabolic rate lower than 0.96 [65].

3.3. Anthropometric measurements

Weight (kg) and height (m) were measured in underwear and barefoot to the nearest 0.1 kg and 0.1 cm, respectively, by trained researchers. BMI was calculated as weight (kg)/height (m^2). Participants were classified into four BMI categories according to the International Obesity Task Force (IOTF) cut-offs for adolescents [102]: equivalent to underweight (<18.5 kg/m²), normal-weight (18.5-24.9 kg/m²), OW (25.0-29.9 kg/m²), and OB (≥30.0 kg/m²). Standard deviation score of BMI (BMI z-score) was calculated using the lmsGrowth method [104]. The cut-off of BMI z-score [544]: underweight (<-2), normal-weight (-2 -1), OW (>1) and OB (>2). Skinfold thickness was measured to the nearest 0.2mm in triplicate [352]. The same trained investigators made all measurements (inter-rater reliability >95 %). BF% was calculated using Slaughter's equations [448]. More details about the anthropometric measurements are given in a previous manuscript [352]. Physical maturations were examined by a physician during a medical examination to determine the pubertal status based on Tanner stages [473]. The final physical maturations were classified into three categories: pre-pubertal (stage 1); pubertal (stage 2 to 4) and post-pubertal (stage 5).

3.4. Blood samples

Blood samples were collected in a randomly selected subsample of the total HELENA-CSS. Adolescents who agreed to be involved in the blood sampling were asked to fast after 8 pm on the previous day. Fasting blood samples, information of adolescents' medical history and recent acute diseases were collected by venipuncture between 8–10 a.m. at schools or hospitals by a medical doctor, A blood sampling questionnaire was completed by the participants for the purposes of assessing fasting status, acute infection, allergies, smoking, vitamin and mineral supplements, and medication. A specific handling, transport and traceability system for biological samples was developed for the HELENA study. All samples were analyzed centrally. The blood sampling procedure has been described elsewhere [188].

3.5. Physical activity

PA was assessed for 7 days by an uniaxial accelerometer (Actigraph GT1M), described previously [338]. At least 3 days of recording with a minimum of 8 hours' registration per day was set as an inclusion criterion. Physical activity, used in the current study, was categorized in the following categories: at least 1 hour of physical activity per day, no physical activity or less than 1 hour of P physical activity per day.

3.6. Statistical analysis

Descriptive data is presented as means with standard deviation or frequency distributions. Energy and total, animal and plant protein intakes were corrected for within person variation using the MSM, which is suitable for estimating population's usual intakes [127]. Statistical differences for total energy and total, animal and plant protein intakes between subgroups (gender and age) were assessed using the Student T-test and ANOVA.

GLM multivariate analysis was used to investigate the associations of indicators (dependent variables) with animal and plant protein intakes, and animal (E%) and plant (E%) energy percentages (independent variables) through three models (stepwise approach): (1) model 1 = unadjusted model; (2) model 2 = model 1 + adjusted for fat intake; (3) model 3 = model 2 + further adjusted for physical activity, confounding factors and interactions, and controlling for the country clustering effect. Potential confounding factors including age (younger group (12.5-14.9 years) and older group (15.0-17.5 years)), gender, tanner stage (pre-puberty, puberty and post-puberty) and two-way interactions between potential confounding factors and independent variables were included in the model 3. The results of the model 3 will be the final statistical results. Anthropometry and serum biomarkers were investigated separately. In addition, animal and plant protein intakes, and the energy percentage (E%) from animal and plant protein were examined in a separate model due to colinearity.

All statistical analysis were performed using the statistical software SPSS for Windows version 18 (SPSS Inc, Chicago, IL, USA). Results were considered statistically significant at α two-tailed level of 0.05.

4. RESULTS

A total of 1804 out of 3528 adolescents (47% males) from 8 centers with valid and complete dietary data and measurements of weight and height were included in the analysis (Table 5.1.1). 74% participants were classified in tanner stage 2–4, including 7% in tanner 2, 24% in tanner 3 and 41% in tanner 4. In total 279 adolescents were classified as OW and OB. Mean BMI z-score for both genders was in the normal weight range. Females had higher BF %, but lower BMI z-score compared to males. Furthermore, higher serum lipid profiles and leptin levels were found in females.

4.1. Total energy and total, animal and plant protein intakes

Median total protein contributing to energy intake was 15.5%. Average total protein intakes exceeded the WHO recommendations (10.0 – 15.0% of the total energy intake) [545] and the estimated average requirements (EAR) and population reference intake (PRI) of the European Food Safety Authority (EFSA) (EAR: 0.66 g/(kg.d) for both genders; PRI: males 0.70-0.74 (g/(kg.d), and females, 0.67-0.72 g/(kg.d)) [157] (Table 5.1.2). All but one adolescent met the EAR, while, fourteen and two adolescents did not reach the WHO recommendations for protein intakes and the PRI, respectively.

Mean total protein intake (384 kcal/d) contributed 15.8% to total energy intake. Mean animal protein intakes were the main contributor (59%) to total protein intakes, as opposed to mean plant protein (Table 5.1.3). Total and plant protein intakes were significantly lower in females and the younger group. Body weight adjusted total protein intakes and E% from total protein were significantly lower in the older group. Total energy, total and animal protein intakes and total protein (E%) were higher in obese adolescents than non-obese ones. More specifically, body weight adjusted total protein intake (g/(kg.d)) was significantly lower in OB, and higher in underweight peers.

Table 5.1.1. Anthropometric characteristics and levels of obesity-related biomarkers in adolescents participating in the HELENA-CSS

	Total	Males	Females
Total participants (n)	1804	855	949
Age (years) (mean (range))	14.7 (12.5-17.4)	14.8 (12.5-17.4)	14.7 (12.5-17.4)
12.5-14.9 (n)	1032	481	551
15.0-17.5 (n)	772	374	398
Tanner Stage (n = 1752)		n (%)	
Tanner 1	9 (0.514)	9 (1.1)	0 (0.0)
Tanner 2-4	1294 (73.9)	614 (74.2)	680 (73.5)
Tanner 5	449 (25.6)	204 (24.7)	245 (26.5)
Weight status (n = 1804) [µ]			
Underweight	142 (7.9)	58 (6.8)	84 (8.9)
Normal-weight	1383 (76.7)	649 (75.9)	734 (77.3)
Overweight	222 (12.3)	114 (13.3)	108 (11.4)
Obesity	57 (3.2)	34 (4.0)	23 (2.4)
Anthropometry		Mean (SD)	
BMI z-score (n = 1804)	0.270 (1.1)	0.358 (1.1)	0.190 (1.0)
BF% (n = 1764)	22.0 (8.6)	18.4 (9.1)	25.1 (6.8)
Biomarkers			
TC (mg/dL) (n = 552)	159.1 (27)	151.9 (24.9)	165.8 (27.1)
TG (mg/dL) (n = 552)	67.6 (31.1)	64.5 (31.5)	70.5 (30.5)
LDL-C (mg/dL) (n = 552)	92.6 (24.2)	89.0 (23.2)	96.0 (24.7)
VLDL-C (mg/dL) (n = 552)	13.5 (6.2)	12.9 (6.3)	14.1 (6.1)
HDL-C (mg/dL) (n = 552)	55.6 (10.3)	53.3 (9.3)	57.8 (10.7)
CRP (mg/L) (n = 524)	1.2 (4.0)	1.5 (5.5)	0.841 (1.3)
Glucose (mg/dL) (n = 552)	90.1 (7.0)	91.9 (7.2)	88.5 (6.4)
Insulin (µIU/mL) (n = 545)	9.5 (6.0)	9.0 (6.6)	10.0 (6.6)
Leptin (ng/mL) (n = 518)	18.5 (21.9)	8.1 (12.9)	27.5 (24.1)

SD, standard deviation; BMI, body mass index; BF%, body fat percentage; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein- cholesterol; VLDL-C, very low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein- cholesterol; CRP, c-reactive protein.

[µ] BMI categories is classified based on the International Obesity Task Force cut-offs, underweight: <18.5 kg/m^2, normal-weight: 18.5-24.9 kg/m^2, overweight:25.0-29.9 kg/m^2, obesity: ≥30.0 kg/m^2.

Table 5.1.2. Percentile of total protein intakes and the number of the subjects below the recommendations of European food safety authority in the European adolescents

Characteristics	N	Total protein (g/d)			Total protein (g/(kg.d))			The number of subjects below the recommendations	
		25%	50%	75%	25%	50%	75%	EAR[µ]	PRI[µ]
Total	1804	76	91	109	1.3	1.6	2.0	1	2
Gender									
Males	855	90	106	127	1.5	1.8	2.3	0	0
Females	949	68	80	94	1.2	1.5	1.8	1	2
Age (years)									
12.5-14.9	1032	74	90	108	1.4	1.7	2.1	1	1
15.0-17.5	772	77	94	112	1.3	1.6	1.9	0	1

EAR: estimated average requirement; PRI: population reference intake.

[µ] EAR: 0.66 g/(kg.d) for both genders; PRI : males, 0.70-0.74 g/(kg.d) and females, 0.67-0.72 g/(kg.d).

4.2. Associations between total, animal and plant protein intakes and cardio-metabolic indicators

Figure 5.1.1 shows a significant decline in BF% across the total protein tertiles ($P < 0.001$) by age. But no significance was observed in males and females. The results of the GLM multivariate analysis showed that crude BF% was inversely associated with absolute animal and plant protein in model 1, but crude BMI z-score and BF% were positively associated with animal protein (E%) (Table 5.1.4). Absolute animal protein intake was inversely associated with crude serum biomarkers including TC, TG, VLDL-C and leptin, but positively with serum fasting glucose. While absolute plant protein intake was inversely associated with crude TC, HDL-C, and leptin, but positively with serum fasting glucose. After adjustments for fat intake (Model 2), BMI z-score became positively associated with absolute animal protein intake, but several significant associations found in model 1 disappeared. Leptin kept to be inversely associated with absolute animal protein intake in model 2, and BF%, TC and HDL-C with absolute plant protein intake. Only serum HDL-C became positively associated with absolute animal protein intake, after further adjusting for confounding factors, physical activity and interaction factors (Model 3). Inverse associations were observed between BMI z-scores and BF%, and absolute plant protein intake. Whereas both BMI z-scores and BF% were positively associated with animal protein (E%). No biomarker was associated with percentage of energy intake derived from animal and plant protein (data not shown).

Table 5.1.3. Estimated means of energy, total, animal and plant protein intakes, and energy percentage of protein intakes of adolescents participating in the in HELENA-CSS stratified by gender, age, tanner and BMI category

Characteristics	N	Energy (kcal/d)	Total protein (g/d)	Total protein (g/(kg.d))	Animal protein (g/d)	Plant protein (g/d)	% energy contributing to total energy intake	
							Total protein	Plant protein
				Mean intake (SD)				
Total	1804	2450 (637)	96 (28)	1.7 (0.6)	58 (23)	38 (13)	15.8 (2.8)	6.2 (1.3)
Gender								
Males	855	2792 (655)	110 (29)	1.9 (0.6)	66 (24)	43 (13)	15.9 (3.0)	6.2 (1.3)
Females	949	2141 (428)*	83 (20)*	1.6 (0.5)*	50 (18)*	33 (10)*	15.6 (2.7)	6.3 (1.3)
Age (years)								
12.5-14.9	1032	2358 (637)	94 (28)	1.8 (0.6)	57 (22)	37 (12)	16.1 (2.9)	6.2 (1.4)
15.0-17.5	772	2752 (713)**	98 (29)**	1.6 (0.5)**	58 (23)	39 (12)**	15.4 (2.8)**	6.2 (1.2)
Weight status								
Underweight	142	2443 (631)	94 (28)	2.2 (0.7)	56 (21)	39 (12)	15.5 (2.7)	6.3 (1.2)
Normal-weight	1383	2458 (635)	96 (28)[a]	1.8 (0.6)[a]	58 (22)	38 (13)	15.7 (2.8)	6.2 (1.3)
Overweight	222	2397 (636)	96 (29)[ab]	1.4 (0.4)[ab]	59 (24)	37 (11)	16.2 (3.0)[b]	6.2 (1.3)
Obesity	57	2476 (701)	102 (33)[abc]	1.2 (0.4)[ab]	63 (27)	38 (12)	16.5 (3.1)[b]	6.2 (1.2)

SD, standard deviation.

* Mean value was significantly different between males and females by Student *T*- test ($P < 0.05$).

** Mean value was significantly different from the young group (12.5-14.9 years) by Student *T*- test ($P < 0.05$).

[a] Mean value was significantly different from underweight by ANOVA, ($P < 0.05$, Bonferroni correction).

[b] Mean value was significantly different from normal-weight by ANOVA, ($P < 0.05$, Bonferroni correction).

[c] Mean value was significantly different from overweight by ANOVA, ($P < 0.05$, Bonferroni correction).

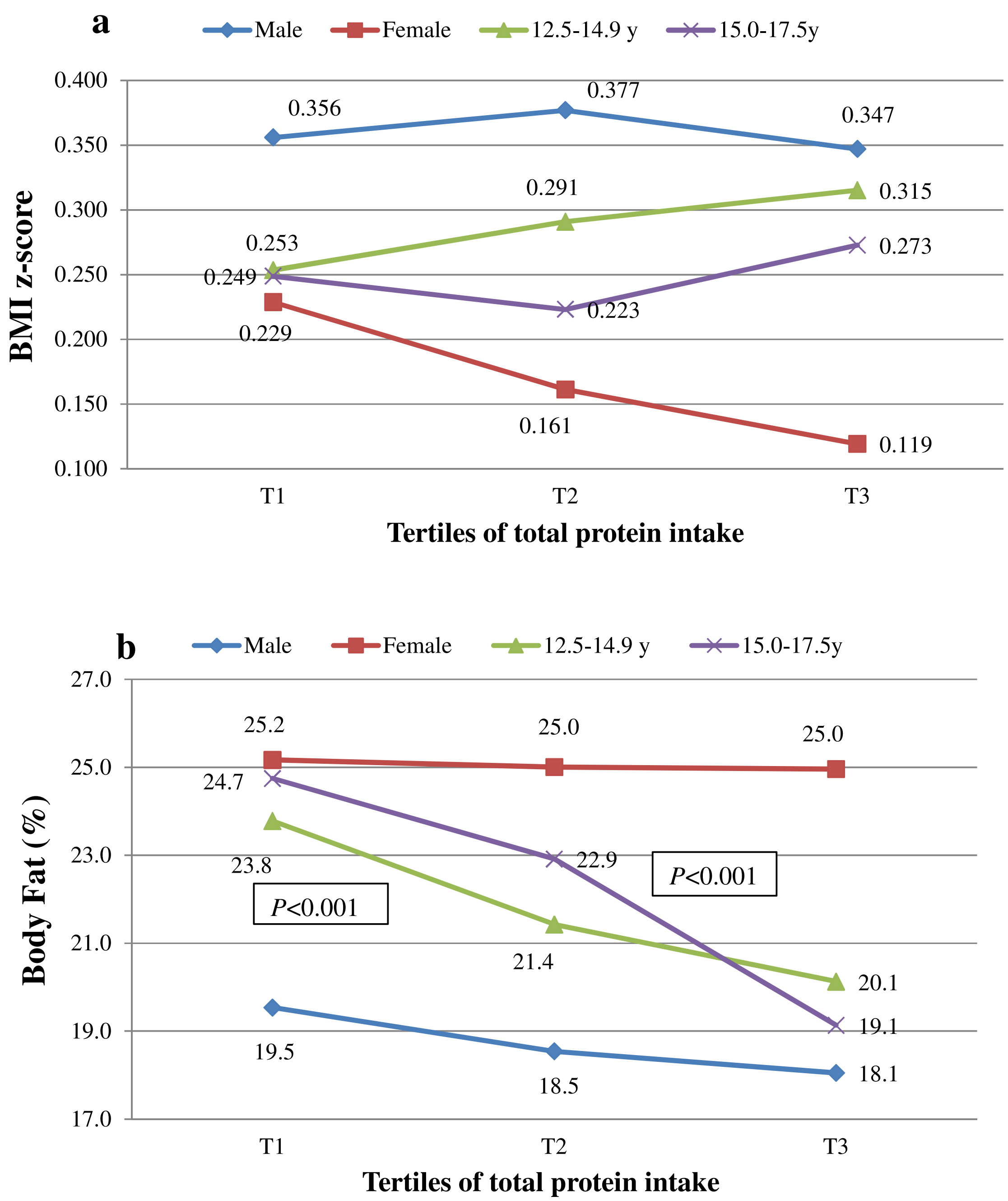

Figure 5.1.1. Tertiles[μ] of total protein intake (g/d) and anthropometric indicators in adolescents participating in HELENA-CSS (n = 1804).

[μ]Tertile 1 (T1): <81 g/d; tertile 2 (T2): 81 g/d to 103 g/d; tertile 3 (T3): ≥103 g/d.

Table 5.1.4. Associations between dietary animal and plant protein intakes (g/d and E%) and cardio-metabolic indicators of adolescents participating in the HELENA-CSS (n = 1804)

Dependent variables[µ]	Animal protein (g/d)				Plant protein (g/d)			
	B	SE	95 % CI	*P*	B	SE	95 % CI	*P*
BMI z-score								
Model 1	0.001	0.001	–0.001, 0.004	0.206	–0.002	0.002	–0.006, 0.002	0.414
Model 2	0.003	0.001	0.000, 0.006	0.023	0.000	0.002	–0.005, 0.004	0.847
Model 3	–0.000002	0.000001	–0.000007, 0.000	0.421	–0.012	0.005	–0.023, –0.001	0.027
Body fat (%)								
Model 1	–0.054	0.009	–0.071, –0.036	<0.001	–0.162	0.016	–0.194, –0.131	<0.001
Model 2	–0.009	0.010	–0.030, 0.011	0.386	–0.106	0.019	–0.144, –0.069	<0.001
Model 3	–0.000052	0.000018	–0.000087, – 0.000016	0.004	–0.139	0.040	–0.217, –0.060	0.001
	Animal protein (E%)				Plant protein (E%)			
BMI z-score								
Model 1	0.021	0.008	0.005, 0.038	0.011	0.012	0.019	–0.026, 0.050	0.533
Model 2	0.021	0.008	0.005, 0.037	0.011	0.008	0.020	–0.031, 0.046	0.692
Model 3	0.024	0.009	0.006, 0.043	0.010	–0.027	0.021	–0.067, 0.013	0.188
Body fat (%)								
Model 1	0.209	0.067	0.077, 0.341	0.002	0.124	0.156	–0.181, 0.429	0.426
Model 2	0.196	0.065	0.068, 0.325	0.003	–0.179	0.154	–0.482, 0.123	0.245
Model 3	0.168	0.070	0.030, 0.305	0.017	–0.229	0.151	–0.526, 0.068	0.130

SE, standard error of coefficient B; CI, confidence interval.

[µ]Model 1, unadjusted; model 2, adjusted for fat intake; model 3, model 2 further adjusted

5. DISCUSSION

The HELENA study is the first large-scale European adolescent population-based dietary survey of 8 European countries providing data on the nutritional intake, status, main determinants of food choices and preferences among European adolescents. The current study is the first to provide information on intakes of total, animal and plant proteins and their associations with OB and cardio-metabolic indicators.

5.1. Total energy and total, animal and plant protein intakes

The contribution of protein to energy intake in our study was similar to that reported in Greek and Italian adolescents, lower than that of Spanish peers (male: 17.2%, female: 17.8%) [432], but higher than adolescents in review studies of Western, Central and Eastern European countries [303;387;412]. In addition, total protein intake was reported to be slightly lower in Italian peers (male: 99 g/d, female: 82 g/d) [433], Spanish males (male: 105 g/d, female: 86 g/d) [432], and Western European adolescents [303;412]. The adolescents in this study had much higher animal and plant protein intakes than those of Belgian peers (male: 52 g/d, female: 37 g/d; male: 30 g/d, female: 24 g/d, respectively) [303] and higher plant protein intake (male: 30 g/d, female: 25 g/d), but lower animal protein intake than Spanish peers (male: 74 g/d, female: 60 g/d).

5.2. Associations between total, animal and plant protein intakes and cardio-metabolic indicators

Obese HELENA participants consumed more total protein than non-obese participants. Evidence from other European studies indicate higher contribution of animal sources[113;387] to total protein and lower from plant protein consumptions [412], which might point to a relationship between increasing prevalence of OB in European adolescents. Our results suggest that increasing total protein intakes may be inversely associated with adolescents' BF%, which can be explained by plant protein intakes being significantly inversely associated with BMI z-score and BF%, after adjustment for fat intake, physical activity and confounding factors. Consistent with our findings, observed benefits of increasing total and plant protein intakes on body composition [72;495] could be attributed to the protein effect on increasing stimulated fat oxidation and building of lean body mass [456]. Conversely, the results of a previous randomized trial on obese adolescents (11–16 years) demonstrated that increasing protein consumption conferred no benefit on weight loss and body composition in the treatment of adolescent OB [413]. The different study design and target population might partly explain differences observed. Remarkably, the level of serum leptin was found to be extremely low among males in our study. High levels of leptin can easily be observed in female adolescents, because leptin was reported to play a critical role in the regulation of puberty, especially in females [425]. Serum leptin is proven to be related to BF% [105], and this might partly explain our finding on why females kept high BF% when increasing total protein intake, whereas BF% in males decreased gradually.

Evidence shows that plant protein from vegetables, fruits, and legumes not only improves body composition, but also results in lower body weight compared to animal protein [14;209]. In our study, although animal protein intake was found to be weakly inversely associated with BF%, animal protein (E%) was observed to be positively associated with BF%. Previous studies concluded that total and animal protein intakes might be responsible for increasing body weight and BMI in adolescents [209;413]. Mirkopoulou *et al.* suggested that extremely high protein intakes, animal protein in particular, might increase the risk of adolescents' OB due to higher energy consumption [337]. Furthermore, the results of a longitudinal study suggested that a high animal protein intake in mid-childhood might be associated with an earlier pubertal growth and spurt peak height velocity, whereas a higher plant protein intake could delay puberty [193]. On the contrary, some studies disagreed the above hypothesis of increased intake of total and animal protein resulting in decreasing the risk of OW and OB [388;472] by affecting the appetite. A randomized 8-weeks parallel intervention trial suggested that seafood protein sources from cod and salmon were efficient to treat OB because of caloric restriction and lower saturated fatty acids intake [388]. Therefore, the amount of total, animal and plant proteins in the diet may be a critical factor on prevention against OW and OB.

Plant protein based diets in childhood could be responsible for lowering the risk of MetS and its consequence in the adulthood [238]. In the current study, only serum HDL-C was found to be weakly positively associated with animal protein intake. The increases in HDL-C might possibly be explained by the inverse association of animal protein intake with BF%. Mirkopoulou *et al.*

reported that no association with blood lipid profile was observed in Greek adolescents [337], supporting most of our results, as similarities in the study design and target population might explain similarities in observations. Some cross-sectional studies showed that plant based diets were associated with more favorable lipid levels in adolescents by lowering TC and LDL-C, but increasing the HDL-C levels [47;154], whereas high intakes derived from animal sources were associated with an increased risk of MetS [155]. However, it has to be considered that adolescence is a critical period with inevitable increases in energy and nutrient intakes to regulate hormone balances resulting in physical, behavior and social development. Leptin is a protein hormone that has a key role in regulating energy intake and energy expenditure, including appetite in the longer term [171;282]. In the current study, no significance of serum leptin was found in model 3, but it was negatively associated with animal and plant protein intakes in model 1 and model 2, respectively. The status of statistical significance between serum leptin and plant protein intake changed in the model 2 compared to model 1 due to fat intake. In addition, fat intake can be a critical factor for the serum lipid profile and plant protein intake. No study has provided evidence on clear mechanisms, though it is possible that plant protein intake might stimulate serum leptin via homeostasis impacting on body weight and BF%. In addition, female, OW and obese adolescents in particular, during puberty might most likely underestimate energy and dietary intakes, which may bias the associations. Confounding factors, such as gender, age, Tanner stage and region, may account for some unexpected findings, serum biomarkers in particular.

5.3. Strengths and limitations

This European nutrition survey is the first large-scale study among European adolescents that used a standardized approach across 8 participating centers. Additionally, it is the first study evaluating total, animal and plant protein intakes in European adolescents stratified by gender and age, and investigating associations with anthropometry and serum biomarkers as studies with the same standardized methodology across European countries are limited.

The current study has also some limitations including the dietary assessment method used to assess diet that only included dietary information of two non-consecutive days. The 24-h dietary recall method does not allow quantifying proportions of non-consumers for particular food items, especially for those less frequently consumed. In order to decrease the influence of such limitation, nutrient intakes were corrected for within- person variability by applying the MSM method. Moreover, accuracy of collected data relies on the individual's ability to remember foods and beverages consumed in the past 24 hours, and might, therefore, be biased towards misreporting. In this respect, the 24-h dietary recalls were performed through computer-assisted HELENA-DIAT software to standardize the recall procedures as much as possible. Food pictures, showing daily foods consumed by European adolescents, were used in order to facilitate the participants to recall the potion size of the foods consumed in the previous days, which assisted participants and interviewers in accurately assessing the consumed amounts. The same food composition table for conversion of food intake data to estimated nutrient intakes was used for all survey centers. In this way, differences in definitions, analytical methods, units and modes of expression were overcome. However, missing foods of protein contents in the BLS table were calculated via recipes or taken from local food composition tables. In addition, the small sample size of serum biomarkers may also be a potential influencing factor leading to weak linear relationship between animal and plant protein intakes and serum biomarkers. Furthermore, the cross-sectional study design of this study cannot assess causality between health outcomes and dietary intakes.

5.4. Recommendations

Protein is critical for the development of bone and muscle mass, and health in adolescents. An increased protein intake is one of the most common approaches to the dietary management of OB and related chronic diseases. However, extra high protein intake can result in side effects due to imbalance in energy intake and food consumption. The findings of current study indicate that plant protein had more protective effect against OB compared to animal protein, although HDL-C was found to be weakly positively associated with absolute animal protein intake. We noticed that participants exceeded protein intake based on WHO requirement, and almost 2/3 sources were from animal origin rather than from plants, which may influence body weight and body composition. The findings of our study highlight that future public health policies and school policies need to be developed and implemented to help establishing healthy food preferences, and adjusting food concepts and dietary behaviors in adolescents. Possible prevention strategies could include the development of multicomponent school-based interventions combining education and environmental changes towards increased intakes of plant proteins from legumes and vegetables.

6. CONCLUSION

The total protein intake of European adolescents exceeded the recommendations and animal proteins contribute most to the energy intake derived from total protein intake. Total and animal protein intake and E% derived from protein intake were higher in obese subjects. A negative association of total protein intake was found with BF%. GLM multivariate analysis indicates inverse associations, on one hand, between BMI z-score and plant protein intake, and on the other hand between BF% and animal and plant protein intakes. Both BMI z-score and BF% were positively associated with animal protein (E%). In conclusion our findings suggest that plant protein intakes may play a role in preventing OB among European adolescents. Further longitudinal studies should be conducted to investigate these potential beneficial effects of plant protein intakes in the prevention of OB and related chronic diseases.

CHAPTER 5.2

Dietary fiber intake and its association with indicators of adiposity and serum biomarkers in European adolescents: the HELENA study

Chapter based on this manuscript:

Lin Y, Huybrechts I, Vereecken C, Mouratidou T, Valtuen˜a J, Kersting M, *et al.* Dietary fiber intake and its association with indicators of adiposity and serum biomarkers in European adolescents: the HELENA study. *Eur J Nutr*.2015;54(5):771-82.

1. ABSTRACT

Purposes

To evaluate total, energy-adjusted dietary fiber (DF), water-soluble fiber (WSF), and water-insoluble fiber (WIF) intakes in European adolescents and to investigate their association with indicators of adiposity and serum biomarkers.

Method

This study, conducted from 2006 to 2007, included 1804 adolescents aged 12.5–17.5 years (47 % males) from eight European cities completing two nonconsecutive computerized 24-h dietary recalls. GLM multivariate analysis was used to investigate associations.

Results

Mean DF intake (20 g/day) of the sample met the European Food Safety Authority recommendation, but was below those of the World Health Organization and of the Institute of Medicine. Total DF, WSF and WIF intakes were higher in males ($P<0.001$), but following energy adjustments significantly higher intakes were observed among females ($P<0.001$). Bread and cereals contributed most to total DF, WSF and WIF intakes, followed by potatoes and grains, energy-dense but low-nutritious foods, fruits and vegetables. Moreover, energy-adjusted WSF and WIF were positively associated with body fat percentage (BF%), waist to height ratio and low-density lipoprotein cholesterol, while energy-adjusted WSF was inversely associated with serum fasting glucose (β = -0. 010, P = 0.020)

Conclusion

Total DF intakes are rather low in European adolescents. An inverse association with serum fasting glucose might indicate a possible beneficial role of DF in preventing insulin resistance insulin resistance and its concomitant diseases, even though DF intakes were positively associated with adolescents' BF%. Therefore, further longitudinal studies should elaborate on these potential beneficial effects of DF intake in the prevention of obesity and related chronic diseases.

2. INTRODUCTION

Evidence indicates that OW and OB during childhood is turning into the biggest epidemic concern of the twenty-first century [44]. Childhood OB is an important predictor of OB in adulthood [151]. In addition, its negative health consequences, i.e., MetS, CVD, T2D, were reported to have a negative effect on life quality and result in higher prevalence of morbidity and mortality in adulthood [309;311;374;401]. In Europe, approximately 14 % of the children and adolescents participating in the KIDSCREEN Health Interview Survey were classified as OW, 51% of those diagnosed with OW were adolescents [377]. Moreover, over 20000 obese European children have been diagnosed with T2D and over 400000 with impaired glucose tolerance [311].

DF classified into WSF and WIF, has been considered a leading dietary factor in the prevention and treatment of OB and its concomitant chronic diseases over the past four decades [20]. DF, as the residue of plant food resistant to hydrolysis by human alimentary enzymes, is a heterogeneous mixture of polysaccharides and lignin, offering potential health benefits to humans [254]. WSF delays small bowel absorption, which can subsequently reduce cholesterol absorption due to viscous solutions in the gastrointestinal tract [471]. In addition, fermentation of WSF can produce gases and short-chain fatty acids causing longer lasting satiety, lowering the glycemic index of foods and retarded absorption due to viscosity effects, consequently, slowing down acute insulin response [324;471]. WIF, on the other hand, can increase the bulkiness of stool and fecal mass, thereby shortening the transit time due to non-digestibility [471].

A significant reduction of DF intake has been observed in industrialized countries paralleling with a dramatic rise of OB [354]. The WHO recommends an intake of 20 g/day of NSP and at least 25 g/day of total DF, which can protect against OB and its consequences [254]. The results of previous studies indicate that European adolescents had lower DF intake compared with the WHO recommendation, mainly due to high intakes from animal sources [113;291;305;412;432;551]. Evidence states that DF intake plays a strong role in the prevention of OW and OB in childhood [7;85;118;406] through weight maintenance and blood lipid regulation as a result of a balanced energy intake [216;471]. Recent US longitudinal studies show that a high consumption of DF was beneficially affecting body composition in children [4;118] and CRP levels [318]. In a dietary intervention in OW or OB adults, great improvements in risk factors of serum biomarkers including fasting lipids, glucose and insulin concentrations were observed in those participants with higher DF intake [380].

Because of lack of comprehensive knowledge of DF, WSF and WIF intakes, and food sources of DF in European adolescents, the purpose of the present study was to assess total, energy-adjusted DF, WSF and WIF intakes in European adolescents participating in the HELENA-CSS. Associations between energy-adjusted DF, WSF, WIF intakes and adiposity-related indicators (BMI z-score, BF%, WHR, waist-to-height ratio (W/H)) and serum biomarkers (TC, TG, LDL-C, VLDL-C, HDL-C, CRP, glucose, insulin and leptin) were examined.

3. METHODS

3.1. Survey population

HELENA-CSS is a European Commission funded project on lifestyle and nutrition among adolescents from 10 European cities: Athens and Heraklion (Greece), Dortmund (Germany), Ghent (Belgium), Lille (France), Pecs (Hungary). Rome (Italy), Stockholm (Sweden), Vienna (Austria), and Zaragoza (Spain), that ran between October 2006 and December 2007. Due to logistical reasons, adolescents from Heraklion and Pecs were excluded from the dietary intake analyses. Male and female adolescents, aged 12.5–17.5 years [divided into the younger age group (12.5–14.9 years) and older age group (15.0–17.5 years)], not participating simultaneously in a clinical trial, and free of any acute infection lasting <1 week before inclusion year were recruited.

A multi-stage random cluster sampling procedure was used to select 3528 adolescents, stratified by geographical location, age and SES. Schools were randomly selected after stratification to guarantee diversity of the sample in culture and SES. Details on sampling procedures, study design and non-respondents have been reported elsewhere [50;341;342]. Participants were included in this study if they had provided complete weight and height measurements and dietary assessment data. The study was approved by the Research Ethics Committees of each city involved. Written informed consent was obtained from the adolescents' parents and the adolescents themselves [48].

3.2. Dietary intake assessment

Two non-consecutive computerized 24-h dietary recalls, instructed by dieticians/researchers, were used to collect the food consumption data. During interviews, adolescents were allowed to ask questions and assistance, and after completion the recall was checked for completeness. Every participant was asked to fill in the HELENA- DIAT twice in a time span of 2 weeks.

HELENA-DIAT is a self-administered computer program based on the YANA-C [504], consisting of a single computerized 24-h dietary recall with a structured program based on six meal occasions (breakfast, mid-morning snack, midday meal, afternoon snack, evening meal and evening snack). The validated YANA-C was designed to obtain a detailed description and quantification of foods consumed, and eventually included more than 800 food items hierarchically organized in 25 food groups, and about 2600 colored photograph sets of more than 300 food items in different portions [503;505]. The 25 original food groups were aggregated into 11 summative ones: (1) beverages (including juices, excluding the rest group); (2) bread and breakfast cereals; (3) potatoes and grains; (4) total vegetables; (5) legumes, soy products, soy drinks; (6) total fruits; (7) milk, milk products, cheese (8) fat, oil, cream cheese, sour cream; (9) meat, poultry, fish, eggs, nut and seeds; (10) rest group defined as energy-dense, low-nutritious foods; (11) miscellaneous.

Dietary data of the HELENA-DIAT were linked to the dietary energy (kcal/day) and DF food composition data (g/day) of the BLS (Bundeslebensmittelschlu¨ssel), version II.3.1, 2005) [161]. DF was defined as sum of all cellulotic polysaccharides, non-cellulotic polysaccharides and lignin. WSF (g/day) was the sum of total pectins, while WIF (g/day) was the sum of cellulose, lignin and hemi-cellulotic polysaccharides. Energy-adjusted DF intake (g/1000 kcal/day) was also calculated using the energy density method [131]. In the present study, under-reporters, defined as individuals with a ratio of energy intake over estimated basal metabolic rate lower than 0.96 [65] were excluded from the study sample for the final data analyses.

3.3. Anthropometric measurements

Weight (kg), height (m), WC (cm) and hip circumference (cm) were measured by well-trained researchers on underwear and barefoot adolescents. Weight was measured with an electronic scale (Type SECA 861. UK) to the nearest 0.1 kg and height in the Frankfort plane with a telescopic height measuring instrument (Type SECA 225, UK) to the nearest 0.1 cm. Thereafter, BMI (kg/m^2) and BMI z-score were calculated. Participants were classified into four BMI categories according to the International Obesity Task Force (IOTF) criteria [103] as follows: underweight (<18.5 kg/m^2), normal-weight (18.5-24.9 kg/m^2), OW (25.0-29.9 kg/m^2), and OB (≥30.0 kg/m^2), which was calculated based on the adjustment of gender and age. Skinfold thickness was measured to the nearest 0.2 mm in triplicate in the left side at biceps, triceps, subscapular, suprailiac, thigh and medial calf with a Holtain Caliper. BF% was calculated from skinfolds thicknesses (triceps and subscapular) using Slaughter’s equations [448]. Waist and hip were measured in triplicate with an anthropometric un-elastic tape SECA 200 to the nearest 0.1 cm and thereafter WHR and W/H were calculated. BMI, BMI z-score and BF% were used as surrogates of total body fat and WHR and W/H as surrogates of central body fat. Identification of physical maturation (stages I–V) was assessed by a medical doctor according to Tanner and White house [473]. More details about the anthropometric measurements are given in a previous manuscript [352].

3.4. Blood sample

Blood samples were collected in a subsample of the total HELENA-CSS. Adolescents involved in the blood sampling were asked to fast after 8 pm on the previous day. In addition, a blood sampling questionnaire was completed by the participants for the purposes of assessing fasting status, acute infection, allergies, smoking, vitamin and mineral supplements, and medication.

A specific handling transport and traceability system for biological samples was developed for the HELENA study. Serum leptin (ng/mL) was measured using the RayBio Human Leptin ELISA (RayBiotech, Norcross, Georgia, USA) kit at UPM (Madrid). All samples were analyzed centrally. The blood sampling procedure has been described elsewhere [188].

3.5. Statistical analysis

Descriptive data are presented as mean with SD, median or frequency distributions stratified by gender and/or age group. Mean dietary intakes were corrected for within-person variation by means of the MSM [127]. The normality of the data and equality of the variances were tested using the Kolmogorov–Smirnov and Levene's test, respectively. The statistical differences of anthropometry, serum biomarkers, total, energy-adjusted DF, WSF and WIS intakes between subgroups (gender and age) were checked by Student's *t* test, median test of nonparametric test, MANCOVA. Results were considered statistically significant at a two-tailed level of 0.05.

GLM multivariate analysis was used to investigate the associations of total and central adiposity indicators (dependent variables) with energy-adjusted DF, WSF and WIF intakes (independent variables), controlling for center clustering effect. The categories of maternal and paternal educational level (lower and lower secondary education, higher secondary education and higher education or university degree; for both maternal and paternal education), physical activity level (at least 1 h physical activity each day, no physical activity or <1 h physical activity each day) and potential confounding factors, including age, gender and tanner stage (pre-puberty, puberty and post-puberty) and two-way interactions were included in the model. Two-way interactions were analyzed between potential confounding factors and independent variables. Indicators of total and central adiposity and serum biomarkers were investigated separately. Serum biomarkers were put in the model after log transformation. The predicted clinical values of biomarkers based on increasing 1 g intakes of energy-adjusted total DF/WSF/WIF have been reported after back log transformation. In addition, energy-adjusted total, WSF and WIF intakes were examined in a separate model due to collinearity.

All statistical analysis was performed using SPSS for Windows version 18.0 (SPSS Inc., Chicago, IL, USA).

4. RESULTS

A total of 1804 out of 3528 adolescents (47 % males) from eight centers with completed, valid dietary information on 2 independent days, were included in the final analysis (Table 5.2.1). Only nine males were classified in Tanner stage 1, with 25 % of total sample in Tanner stage 5. In total, 279 adolescents were classified as OW and OB (12 and 3 %, respectively). Females had higher BF%, serum lipid values and leptin concentrations than males, but lower BMI z-score, WC and WHR compared to males (Table 5.2.1). Furthermore, female adolescents had higher serum lipid profiles and leptin concentrations than males

4.1. Total energy and dietary fiber intake

Total energy intake among European adolescents was 2450 kcal/day (range 1053–5472 kcal/day). Mean total DF intake was 20 g/day (range 8.4–84 g/day), and mean energy-adjusted DF intake was 8.4 g/1000 kcal (3.3–21 g/1000 kcal/day). Additionally, mean WSF and WIF intakes were 6.5 g/day (2.8–34 g/day) and 14.5 g/day (5.7–49 g/ day), respectively (Table 5.2.2). Males had significantly higher intake of energy, DF, WSF and WIF (P<0.001 for all), but lower energy-adjusted DF intakes than females (P<0.001). Older adolescents had significantly higher intakes of DF, WSF and WIF than their younger peers (Ps ≤ 0.003).

Furthermore, OW and obese females consumed less mean total and energy-adjusted DF intakes compared to underweight and normal-weight peers (Figure 5.2.1). No significant differences were observed in total and energy-adjusted DF intakes among BMI categories in males and in the age groups.

Total and energy-adjusted DF intakes were compared to international guidelines (Table 5.2.3). Mean DF intake was on average 20 g/day, which is below the recommendation of WHO [551] and of IOM [234] with only 20 and 2.5% of the participants meeting the recommendations, respectively. Whereas approximately 80% of the adolescents met the recommendations proposed by the EFSA, only 7.5% exceeded 30 g/day (103 males and 33 females) [158]. Concerning energy-adjusted DF intake (8.4 g/1000 kcal/day), 74% adolescents were in line with the EFSA recommendation [158]. In general, fewer OW and obese adolescents met the total DF intake requirements of WHO, EFSA and IOM, compared to the underweight and normal-weight peers.

Table 5.2.1. Anthropometric characteristics and serum biomarkers of adolescents participating in the HELENA-CSS

Items	Total (n=1804)		Males (n=855)		Females (n=949)	
Tanner stage (n=1752)			%			
Tanner 1	0.514		1.1		0.000	
Tanner 2-4	73.9		74.2		73.5	
Tanner 5	25.6		24.7		26.5	
BMI category (n=1804)						
Underweight	7.9		6.8		8.9	
Normal-weight	76.7		75.9		77.3	
Overweight	12.3		13.3		11.4	
Obesity	3.2		4.0		2.4	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	14.7	1.2	14.8	1.3	14.7	1.2
Anthropometry (n=1804)[a]						
BMI z-score	0.270	1.1	0.358	1.1	0.190*	1.0
BF%	22	8.6	18.4	9.1	25.1*	6.8
WC (cm)	71	7.9	72.7	8.3	69.5*	7.1
WHR	0.626	0.084	0.664	0.088	0.591*	0.061
W/H	0.429	0.045	0.429	0.046	0.429	0.045
Serum biomarkers[a]			Median			
TC (mg/dL) (n=552)	158		151		166.0*	
TG (mg/dL) (n=552)	60		58		61	
LDL-C (mg/dL) (n=552)	92		89		94.0**	
VLDL-C (mg/dL) (n=552)	12		11.6		12.2	
HDL-C (mg/dL) (n=552)	55		52		57.0*	
CRP (mg/L) (n=524)	0.682		0.671		0.692	
Glucose (mg/dL) (n=552)	90		91		88.0*	
Insulin (μIU/mL) (n=545)	8.2		7.8		8.6**	
Leptin (ng/mL) (n=518)	10		3.6		18.7*	

SD, standard deviation; BMI, body mass index; BF%, body fat percentage; WC, waist circumference; WHR, waist to hip ratio; W/H, waist to height ratio; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein- cholesterol; VLDL-C, very low-density lipoprotein- cholesterol; HDL-C, high-density lipoprotein- cholesterol; CRP, c-reactive protein

*Mean/median value was significantly different between genders, $P \leq 0.001$, Student *t*-test.

**Mean/median value was significantly different between genders, $P<0.05$, Student *t*-test.

[a]Statistical difference was used Student's t test for mean value of anthropometry and median test of nonparametric test for serum biomarkers.

Table 5.2.2. Estimated mean total energy intake, and total, energy-adjusted, water soluble and water insoluble dietary fiber intakes of adolescents participating in the HELENA-CSS stratified by gender and age category (n=1804)

	Total		**Males**		**Females**		***P****	**12.5-14.9 y**		**15.0-17.5 y**		***P*****
Intakes [a]	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**		**Mean**	**SD**	**Mean**	**SD**	
Energy (kcal/d)	2449.6	636.6	2792.2	655.1	2140.9	427.8	<0.001	2358.4	572.8	2571.5	695	<0.001
Total fiber (g/d)	20.3	6.4	21.7	6.8	19	5.8	<0.001	19.7	6.4	21	6.4	0.002
Energy-adjusted fiber (g/1,000kcal/d)	8.4	2.0	7.8	1.7	8.9	2.1	<0.001	8.4	2.0	8.3	2.0	0.074
Water-soluble dietary fiber (g/d)	6.5	2.1	7.0	2.1	6.1	1.9	<0.001	6.4	2.1	6.8	2.0	0.001
Water-insoluble dietary fiber (g/d)	14.5	4.6	15.6	5	13.6	4.1	<0.001	14.1	4.5	15	4.7	0.003

SD, standard deviation

*Mean value was significantly different between genders

**Mean value was significantly different between age categories

[a] Multiple analyses of covariance (MANCOVA) (*P*<0.05, Bonferroni)

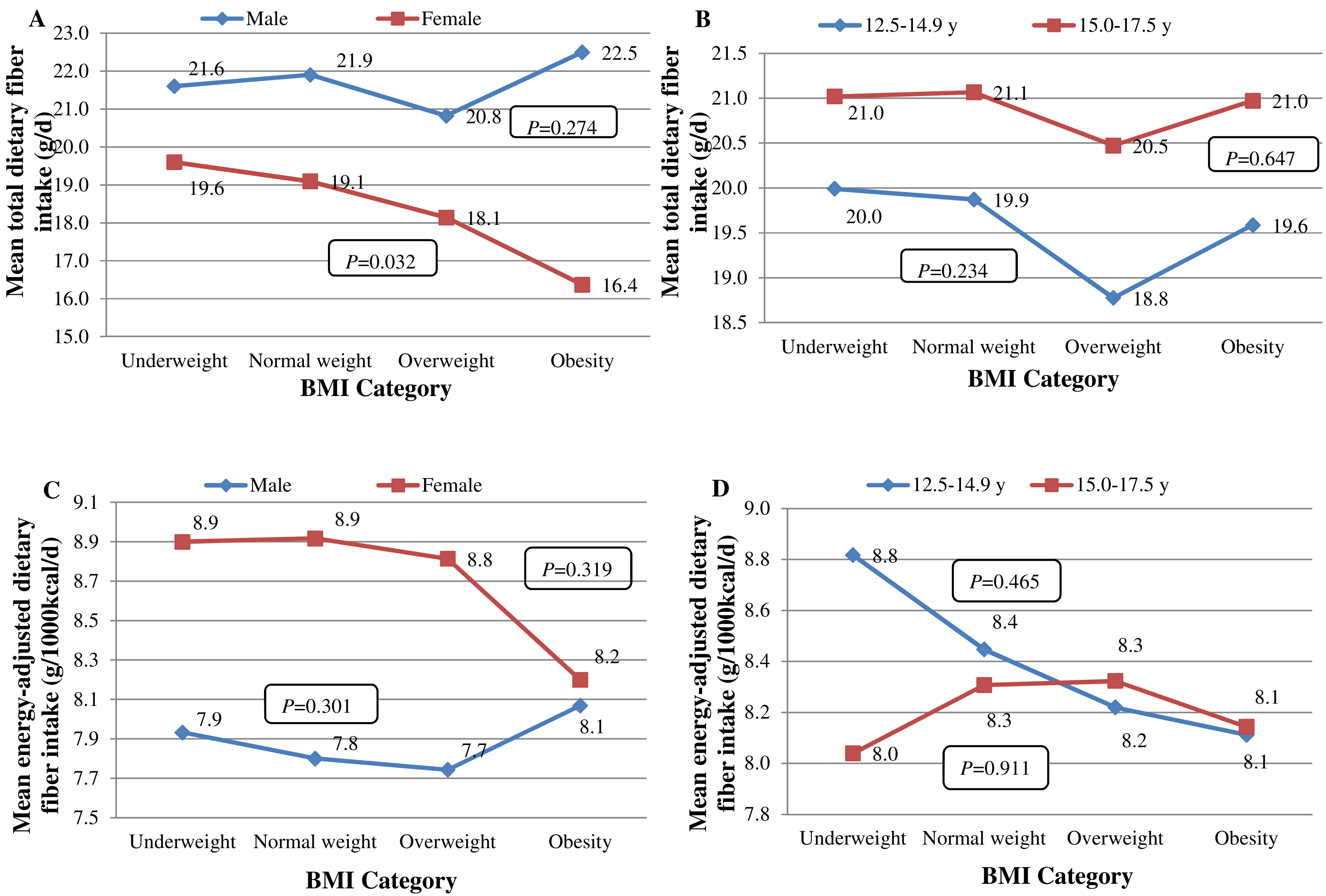

Figure. 5.2.1 Mean (a, b) total and (c, d) energy-adjusted dietary fiber intakes by BMI category stratified by gender (a, c) and age (b, d) of adolescents participating in the HELENA-CSS

Table 5.2.3. Proportion of adolescents participating in the HELENA-CSS meeting the international recommendations for total and energy-adjusted fiber intake (n= 1804)

Organization	Recommendation	Intakes in line with the recommendation (%)						
		All	Males	Females	Underweight	Normal-weight	OW	OB
WHO	Total fiber: 25 g/d	20.0	27.8	14.5	19.0	20.8	15.3	22.8
EFSA	Total fiber:15-30 g/d	80.0	84.8	83.9	84.5	79.5	77.5	78.9
EFSA	Energy-adjusted fiber: 7.1-10.5 g/1000kcal/d	73.6	62.8	93.2	75.4	73.0	75.7	77.2
IOM	Males total fiber: 38 g/d	NA	2.1	NA	1.7	1.8	2.6	5.9
IOM	Females total fiber: 26 g/d	NA	NA	11.4	11.9	11	5.6	0.0

WHO, World Health Organization; EFSA, European Food Safety Authority; IOM, Institute of Medicine; OW, overweight; OB, obesity; NA, Not available

4.2. Food groups contributing to total, water-soluble and water-insoluble dietary fiber intakes

The main food groups contributing to total DF, WSF and WIF intakes in the total population, and among males and females consisted of bread and cereals (bread and rolls in particular), followed by potatoes and grains, rest group products, fruits and vegetables (Table 5.2.4). The main contributing food subgroups were the fruit subgroup including fresh fruits, fruit salad and processed fruit, the vegetable subgroup including fresh vegetables, vegetable salad, prepared vegetables excluding potatoes, and the potatoes and grains subgroup including starch roots, potatoes contributing more among females; while among males, bread and rolls, pasta, breakfast cereals, rice and other cereals contributed relatively more.

4.3. Adiposity indicators and serum biomarkers

Further investigation shows that, on the one hand, BF% was positively associated with energy-adjusted WSF and WIF intakes (B = 1.7, P = 0.005; B = 0.706, P = 0.014, respectively), and W/H and LDL-C positively with energy-adjusted WSF intakes (B = 0.009, P = 0.014; B = 0.031, P = 0.047); on the other hand, serum fasting glucose was inversely associated with energy-adjusted WSF intake, respectively (B = -0.010, P = 0.020). With increasing 1 g intakes of energy-adjusted WSF/WIF, BF% could increase by 1.7/0.706%, respectively, W/H by 0.009 ratio unit and LDL-C by 0.031 mg/dL with 1.03 mg/dL predicated clinical value; while with 1 g energy-adjusted WSF intake, serum fasting glucose could reduce 0.010 mg/dL with 0.990 mg/dL predicated clinical value. Total energy-adjusted fiber intake was not associated with any adiposity and biomarker parameters (Table 5.2.5).

Table 5.2.4. The contributions (%) of consumption of different food groups to total , water-soluble and water-insoluble dietary fiber intakes

Food Groups [a]	Rank	Total Fiber			Water-soluble dietary fiber			Water-insoluble dietary fiber		
		All	Males	Females	All	Males	Females	All	Males	Females
Beverages (including juices, excluding the rest group)		3.0	3.0	3.1	3.5	3.5	3.5	2.8	2.8	2.8
Bread and breakfast cereals		24.5	25.8	23.7	27.4	28.8	26.8	22.2	23.5	21.4
Bread and rolls	1	20.8	21.6	20.5	24.4	25.3	24.1	18.4	19.2	18.1
Breakfast cereals	8	3.7	4.2	3.3	3	3.4	2.7	3.7	4.3	3.3
Potatoes and grains		17.5	18.0	16.9	20.1	20.6	19.4	17.9	18.3	17.4
Starch roots, potatoes	6	6.7	6.4	7.0	6.9	6.5	7.2	7.1	6.8	7.4
Pasta	4	7.7	8.0	7.3	9.3	9.5	8.9	8.0	8.3	7.7
Total vegetables		10.0	9.0	11.0	9.6	8.8	10.3	10.2	9.0	11.2
Fresh vegetables, vegetable salad excluding potatoes	3	9.8	8.8	10.7	9.3	8.6	10.0	10.0	8.9	11.0
Legume, soy products, soy drinks		3.8	3.3	4.0	5.4	4.7	5.7	2.8	2.5	3.0
Pulses (excluding fresh peas, sweet corn and broad bean)	10	2.7	2.8	2.4	3.8	4.0	3.2	2.0	2.1	1.8
Total fruits		14.1	12.7	15.4	13.3	12.1	14.4	14.1	12.8	15.5
Fresh fruits, fruit salad, processed fruit	2	13.9	12.6	15.1	13.1	12.0	14.2	14.0	12.6	15.3
Milk, milk products, cheese		3.9	4.0	3.7	1.9	2.0	1.8	4.7	4.8	4.5
Fat, oil, cream cheese, sour cream		0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3

Meat, poultry, fish, eggs, nut and seeds		4.5	4.5	4.5	5.2	5.1	5.2	4.2	4.2	4.2
Meat, poultry and processed meat	9	2.9	3.2	2.5	3.1	3.6	2.6	2.8	3.1	2.4
Rest group (snacks and desserts) [b]		16.7	17.4	16.2	11.5	11.9	11.2	19.1	19.9	18.6
Cakes, pies, biscuits	6	6.3	6.1	6.4	6.5	6.3	6.6	6.4	6.2	6.6
Chocolate	7	5.5	6.1	5.0	1.8	2.0	1.6	7.2	7.9	6.6
Miscellaneous		1.7	2.0	1.2	1.8	2.2	1.4	1.7	2.0	1.2

[a] Top 10 food subgroups contributed to most total fiber intake. The food groups and subgroups: 1. Beverages (including juices, excluding the rest group) including subgroups of (a) water, (b) soups and bouillon, (c) coffee and tea, (d) fruit and vegetable juices, (e) carbonated/soft/isotonic drinks including non-alcoholic wine, non-alcoholic beer, (f) beer, (g) wine and cider, (h) other alcoholic beverages; 2. Bread and breakfast cereals including subgroups of (a) bread and rolls, (b) breakfast cereals; 3. Potatoes and grains including subgroups of (a) starch roots and potatoes, (b) pasta, (c) rice and other cereals, (d) flour; 4. Total vegetables including subgroups of (a) fresh vegetables, vegetable salad, prepared vegetables excluding potatoes, (b) meat substitutes and vegetarian products; 5. Legume, soy products, soy drinks including subgroups of (a) pulses (excluding fresh peas, sweet corn and broad bean), (b) soya beverages, (c) desserts and puddings soya based; 6. Total fruits including subgroups of (a) fresh fruits, fruit salad, processed fruit, (b) olives and avocado; 7. Milk, milk products, cheese including subgroups of (a) white milk and buttermilk, (b) yogurt and fromage blanc (quark), (c) milk and yogurt beverages, (d) cheese (excluding fromage blanc (quark)), (e) desserts and puddings milk based (including ice cream), (f) other milk products; 8. Fat, oil, cream cheese, sour cream including subgroups of (a) butter and animal fats, (b) margarine and lipids of mixed origins; 9. Meat, poultry, fish, eggs, nut and seeds including subgroups of (a) meat, poultry and processed meat, (b) fish products, (c) eggs, d. nuts and seeds (including nut- and seed spreads), (e) nuts, seeds and olives; 10. Rest group including subgroups of (a) cakes, pies, biscuits, (b) savoury snacks, (c) chocolate, (d) creams (including non-dairy and coffee creams), (e) sugar, honey, jam and syrup, (f) confectionery non chocolate, (g) other sugar products, (h) sauces (excluding dessert sauces), (i) products for special nutritional use; 11. Miscellaneous

[b] Rest group (snacks and desserts) was defined as energy-dense, low-nutritious foods

Table 5.2.5. Associations between indicators of adiposity and serum biomarkers, and energy adjusted total, water-soluble and water-insoluble dietary fiber intakes of adolescents participating in the HELENA-CSS

Dependent variables	Energy adjusted total dietary fiber[a]					
	B	**SE**	**95 % CI**		**The predicted clinical value[c] (95% CI)**	***P***
Body composition						
BMI z-score	0.024	0.031	-0.037	0.086	0.024 (-0.037 to 0.086)	0.436
BF%	0.355	0.233	-0.102	0.812	0.355 (-0.102 to 0.812)	0.127
WHR	0.000	0.002	-0.004	0.005	No change	0.824
W/H	0.001	0.001	-0.002	0.003	0.001 (-0.002 to 0.003)	0.598
Serum biomarkers[b]						
TC	0.001	0.002	-0.003	0.005	1.00 (0.997 to 1.01)	0.669
TG	0.004	0.006	-0.008	0.015	1.00 (0.992 to 1.02)	0.531
LDL-C	0.006	0.004	-0.001	0.013	1.01 (0.999 to 1.01)	0.101
VLDL-C	0.004	0.006	-0.008	0.015	1.00 (0.992 to 1.02)	0.531
HDL-C	-0.002	0.003	-0.007	0.003	0.998 (0.993 to 1.00)	0.397
CRP	-0.016	0.017	-0.050	0.018	0.016 (-0.049 to 0.018)	0.347
Glucose	0.000	0.001	-0.002	0.002	1.00 (0.998 to 1.00)	0.687
Insulin	-0.014	0.007	-0.028	0.000	0.986 (0.972 to 1.00)	0.056
Leptin	-0.012	0.013	-0.037	0.014	0.988 (0.964 to 1.01)	0.375
	Energy adjusted water-soluble dietary fiber[a]					
Body composition						
BMI z-score	0.157	0.082	-0.004	0.319	0.157 (-0.004 to 0.319)	0.055
BF %	1.7	0.608	0.508	2.9	1.7 (0.508 to 2.9)	0.005
WHR	0.003	0.006	-0.008	0.014	0.003 (-0.008 to 0.014)	0.543
W/H	0.009	0.003	0.002	0.015	0.009 (0.002 to 0.015)	0.014
Serum biomarkers[b]						
TC	0.010	0.010	-0.009	0.029	1.01 (0.991 to 1.03)	0.291
TG	0.040	0.025	-0.009	0.089	1.04 (0.991 to 1.09)	0.106
LDL-C	0.031	0.016	0.000	0.062	1.03 (1.00 to 1.06)	0.047
VLDL	0.040	0.025	-0.009	0.089	1.04 (0.991 to 1.09)	0.106
HDL-C	-0.008	0.011	-0.029	0.014	0.992 (0.971 to 1.01)	0.479
CRP	-0.021	0.074	-0.167	0.124	0.021 (-0.154 to 0.132)	0.772
Glucose	-0.010	0.004	-0.018	0.002	0.990 (0.982 to 1.00)	0.020

Insulin	-0.017	0.031	-0.078	0.044	0.983 (0.925 to 1.04)	0.577
Leptin	-0.036	0.055	-0.145	0.073	0.965 (0.865 to 1.08)	0.513
				Energy adjusted water-insoluble dietary fiber[a]		
Body composition						
BMI z-score	0.054	0.039	-0.022	0.129	0.054 (-0.022 to 0.129)	0.165
BF %	0.706	0.287	0.143	1.3	0.706 (0.143 to 1.3)	0.014
WHR	0.000	0.003	-0.005	0.005	No change	0.971
W/H	0.002	0.002	-0.007	0.002	0.002 (-0.007 to 0.002)	0.316
Serum biomarkers[b]						
TC	0.001	0.003	-0.005	0.006	1.00 (0.995 to 1.01)	0.804
TG	0.006	0.007	-0.009	0.021	1.01 (0.991 to 1.02)	0.431
LDL-C	0.007	0.005	-0.002	0.017	1.01 (0.998 to 1.02)	0.118
VLDL	0.006	0.007	-0.009	0.021	1.01 (0.991 to 1.02)	0.431
HDL-C	-0.004	0.003	-0.010	0.003	0.996 (0.990 to 1.00)	0.251
CRP	-0.014	0.022	-0.058	0.029	0.014 (-0.056 to 0.029)	0.519
Glucose	-0.001	0.001	-0.004	0.001	0.999 (0.996 to 1.00)	0.410
Insulin	-0.015	0.009	-0.033	0.003	0.985 (0.968 to 1.00)	0.105
Leptin	-0.012	0.016	-0.045	0.020	0.988 (0.956 to 1.02)	0.455

SE, standard error of coefficient B; CI, confidence interval

[a]In the model, analysis was controlled for country clustering, the categories of maternal and paternal education level (lower and lower secondary education, higher secondary education and higher education or university degree; for both maternal and paternal education), physical activity level (at least 1 hour physical activity each day, no physical activity or less 1 hour physical activity each day) and potential confounding factors including age, gender and tanner stage, and two-way interactions between potential confounding factors and energy adjusted water-soluble / water-insoluble dietary fiber (separate model).

[b]Serum biomarkers were included in the model after log transformation.

[c]The predicted clinical values of biomarkers were calculated after back log transformation based on increasing 1 g intakes of energy-adjusted total dietary fiber/water-soluble dietary fiber/ water-insoluble dietary fiber.

5. DISCUSSION

5.1. Dietary fiber intakes

This is the first large European adolescent population based dietary survey to provide data about total DF, energy-adjusted DF, WSF and WIF intakes among European adolescents. Total DF intake in the study was below the WHO and IOM recommendations, but met the EFSA criteria. Rolland-Cachera *et al.* [412] suggested insufficient DF intakes in adolescents from Western Europe, which is in line with recent research on DF intake in Belgian adolescents [305]. This could be attributed to the more westernized style of eating: our data suggest that European adolescents have higher consumption of animal sources and energy-dense, low-nutritious foods, and lower consumption of vegetables and fruits. These findings are in accordance with the results reported from a study conducted in Southern Europe showing high levels of total and saturated fat intakes [113]. Similarly, OW and obese Swiss children and young adolescents consume more dairy and meat products, but much less vegetables compared with normal-weight peers [3], a trend which was also observed in Spanish adolescents [376]. Total DF intake of the adolescents participating in HELENA was higher than in Belgian (male: 17.8 g/day; female: 15.0 g/day) and US (13.7 g/day) adolescents, due to lower vegetable and fruit intakes in both Belgian and US populations [305;373]. The Asian dietary style is known to consume more foods derived from plant sources compared to the Western style. Additionally, higher energy-adjusted DF intakes were reported in our study than that of Japanese adolescents (12–15 years) (5.6 g/1000 kcal/day for both normal-weight and OW males; 5.8–6.0 g/1000 kcal/day for normal-weight and OW females) [347] and Chinese children aged 7–17 years old (normal-weight: 5.4 g/1000 kcal/day; OW: 5.0 g/1000 kcal/day) [298]. The ratio of WIF and WSF intakes was lower in our study (2.2 for both genders) than in Japanese adolescents (3.7 for both genders), however, both mean WSF and WIF intakes were higher than those estimated in the whole Japanese population in 1998 (3.2 and 11.2 g/day, respectively) [354]. Different standards for food portion sizes due to different cultures and differences in food composition can result in the diversity, and as well as the ranking of contributors to the dietary intakes may be a possible factor. Additionally, observed variations might be attributed to differences in dietary assessment methodology, regional dietary habits, population characteristics and use of food composition tables and regional dietary habits.

In the present study, the most important contributors to DF, WSF and WIF intakes were bread and cereals, followed by rest group products, potatoes and grains, fruits and vegetables. Similar food groups were identified as the major contributors in Belgian (15–18 years) [305] and Italian (mean age: 17 years) adolescents [291] using two repeated, non-consecutive 24-h dietary recalls, and three times 4-consecutive-day 24-h dietary records, respectively. Considering the sub-contributors, legume was one of the main contributors in Italian [291] and US children (2–18 years, in-person 24-h dietary recall) [260], but not for European adolescents participating in the HELENA study. Likewise, rice/wheat and vegetables were top two sub-contributors reported in the Chinese children [298]. Additionally, findings show that energy-dense, low-nutritious foods were consumed in higher amounts than healthy foods such as vegetables and fruits in adolescents.

5.2. Associations between dietary fiber intakes and indicators of body composition and serum biomarkers

DF has been used in the prevention or treatment of OB in children and adults [514]. According to our results, OW adolescents had lower total, energy-adjusted DF intakes than underweight and normal-weight and normal-weight participants, females in particular. Aeberli *et al.* [3] reported that total DF intake was significantly lower in Swiss OW boys, but not in girls. The tendency of total DF intake throughout the weight groups, except females, shows that OB adolescents had the highest consumption of DF. However, differential misreporting (e.g., more underreporting among the obese) could bias those results.

No associations were observed in the present study between body composition indicators and energy-adjusted total DF. Previous studies show an inverse association between DF intake and BMI in healthy female adolescents [4] and central adiposity in OW adolescents [118;386]. However, some studies found no associations of high DF intakes with change of body weight and composition [55;97]. Previous findings reported by Cheng *et al.* [97] stated that DF intake might not affect the development of BF% or BMI during puberty. Likewise, a non-significant change in BF% and BMI upon high consumption of whole-grain derived DF in adolescents was described [97]. However, positive associations of BF% and W/H were found with WSF and WIF. These results are in conflict with other studies indicating that a high consumption of WSF, including fruit- and vegetable-derived DF [318;349;427] and WIF, such as cereal-derived DF

[175;318;406;424;570], not only positively associated with benefits body composition, but also improves blood lipids, glucose and insulin sensitivity due to low-glycemic index diets. Serum fasting glucose concentration was inversely associated with energy-adjusted WSF intake in our study, which is in line with the above indications. However, LDL-C was found positively associated with energy-adjusted WSF intake, while evidence shows that increasing the consumption of DF may protect against high serum TC, TG, LDL-C and CRP concentrations, and improve glucose concentrations in adults [216;318;346]. Consistent with our findings, low DF intakes may be associated with insulin resistance among 8- to 10-year-old and 14- to 16-year-old Danish girls [281]. Conversely, one randomized clinical trial involving children, aged 5–17 years, with hypercholesterolemia showed that cereal- and water-soluble psyllium-derived DF intakes did not improve blood lipid profiles [126], unlike the results of Anderson *et al*. [19] in hypercholesterolemic ambulatory adults. A review study concluded that fruit- and vegetable-derived DF intakes do not directly reduce the risk of OW and OB, whereas WIF does directly reduce the risk of OW and OB [259]. Although total DF, WSF and WIF intake did not have strong associations with body composition and biomarkers, high DF consumption during adolescence was suggested to result in adjustment of blood lipid profile, improvement of insulin resistance and lower SBP during young adulthood [134].

It is noteworthy that physical activity was a very critical confounder as the relationship of DF intakes with adiposity indicators and serum biomarkers was modified after adjusting for physical activity levels (data not shown). Potential confounding factors, such as Tanner stage and region, may explain the effect of DF intake on the contradictory outcome measurements. The small sample size for blood sample collections involved in the current study may also be a potential influencing factor. Although statistical analyses in the current study were adjusted for the Tanner stage, center, age, gender and physical activity, it is likely that the observed weak linear relationship of serum biomarkers with DF intakes may be caused by minor variation of misreporting. Puberty in particular may bias the effect of DF intake on OB-related parameters [4;55;97].

5.3. Strengths and limitations

HELENA-CSS is the first large-scale survey assessing nutrition related aspects among European adolescents, via standardized procedures. Furthermore, it is the first study evaluating total and energy-adjusted DF, WSF and WIF intakes in European adolescents, in relation to Adiposity related indicators, including adiposity and serum biomarkers. Standardized procedures were used to estimate the adolescent's dietary intakes by means of duplicate computer-assisted 24-h dietary recalls. Also all other lifestyle indicators and biomarkers were collected via standardized procedures.

Nonetheless, some limitations of this study need to be considered. A first limitation of the method used is, however, that only information of 2 days was collected. The 24-h dietary recall method does not allow accurate assessments of infrequently consumed foods. In order to correct for such errors, nutrient intakes were corrected for within-person variability by applying the MSM method. Moreover, accuracy of collected data relies on the individual's memory in the past 24 h and might, therefore, be biased toward underreporting. In this respect, the 24-h dietary recalls were performed through computer-assisted HELENA-DIAT software to standardize the recall procedures as much as possible.

Since DF definitions differ between the different local food composition tables and due to many missing data of fiber content in these tables, the same food composition table for conversion of food intake data to estimated nutrient intakes was used for all survey centers. In this way, differences in definitions, analytical methods, units and modes of expression were overcome. In this regard, the dietary data of the HELENA-DIAT were linked to BLS [161]. Data from each country were linked to this database to ensure standardization of available measures. However, DF contents of missing foods in the BLS table were calculated via recipes or borrowed from local food composition tables. Second, the small sample size of serum biomarkers could result in weak linear relationships between DF intakes and serum biomarkers. Additionally, we did not consider food processing, which may have influenced the accuracy of total, WSF and WIF estimates.

6. CONCLUSION

In this first large-scale nutrition survey among European adolescents, average total and energy-adjusted DF intakes met the EFSA recommendations, but were below the WHO and IOM recommendations. The food group of bread and cereals was the most important contributor to total DF, WSF and WIF intakes, followed by rest group products, potatoes and grains, fruits and vegetables. Furthermore, our results indicate that energy-adjusted WSF and WIF are positively related to BF%, W/H and LDL-C, but inversely related to serum glucose. Although few associations were found, WSF and WIF may play a beneficial role in DF by preventing insulin resistance and its concomitant diseases, due to low-glycemic index diets. However, DF intakes can be positively related to adolescents' BF%. Further longitudinal studies should carry on these potential effects of DF intake in the prevention of OB and/or related chronic diseases.

CHAPTER 6

RESULTS FROM CHINESE POPULATION

A cross-sectional study assessing protein and dietary fiber intakes in some Chinese regions with different types of diet and their association with overweight and obesity

1. ABSTRACT

Objectives

To assess and compare total, animal & plant protein, and dietary fiber (DF) intakes between gender-age and dietary region-age subgroups in China and to investigate how food group-specific protein and fiber intakes relate to obesity.

Methods

The China Health and Nutrition Survey- cross-sectional study (2004) collected dietary information using three consecutive 24-h dietary recalls. 9720 participants from the nine provinces (≥3 years, 48.7% men) completed all interviews, stratified in three gender-age groups: preschoolers, school-aged children, and adults; and five diet regions: heavy meals, sweet but less heavy meals (SM), hot and spicy meals, flour (pasta)-rich meals, and variety meals. BMI and waist circumference (WC) were used as measurements of body composition.

Results

73.8% of the proteins were from vegetable origin. Cereals were the main contributor to total protein intakes and vegetables to fiber intakes. However, total protein and DF intakes were below the Chinese dietary guidelines. Relatively unhealthy body composition (higher BMI and WC) was observed in the SM region with the highest total and animal protein intakes. In adults, dairy-derived protein, and the intakes of protein and DF from cereal and vegetable were inversely associated with both BMI and WC, whereas the intake of proteins and DF through the consumption of fish and legumes were positively related with obesity indicators. Few associations were found among preschoolers and school-aged children.

Conclusions

Our results indicate that total protein and DF intakes were inadequate for the population living in the participating regions. Relatively high animal protein intakes and reduced plant based diets might be related to increased obesity prevalence in China.

2. INTRODUCTION

OW and OB in children and adults have been considered an epidemic public health problem since the past three decades [141]. Although the prevalence of OB is still relatively low in China as compared to developed countries, a rapid increase has been observed concomitant with the economic growth and urbanization [556;560]. Over the past 30 years, the prevalence of OB in Chinese adults increased from 2.9% to 11.4% among men and from 5.0% to 10.1% among women [560]. The prevalence of abdominal OB increased from 19.3% among men and from 18.1% among women [560]. In 2011, the prevalence of OW and OB in children and adolescents, aged 6 to 19 years old, living in big cities such as Beijing and Shanghai was reported 17.7% and 14.4%, respectively, which is close to the prevalence of children in the Western countries [556].

Dietary intake is a crucial factor for the development of OW and OB. The traditional Chinese diet is rich in grains, vegetables, fruits and few animal products. However, the dietary pattern has changed steadily over the last decades due to the nutrition and lifestyle transition towards a Westernized diet pattern and lifestyle [573;574]. As a result, the overall energy and protein intake from animal sources increased considerably, whereas the consumption of cereals, and fruits and vegetables declined gradually [140;573]. Moreover, regions with different types of diet can be distinguished within China as a result of geography and climate, but also social and cultural differences [437]. For example, in the North-eastern part including the provinces of Liaoning and Heilongjiang, people rely heavily on preserved foods due to the harsh winters and relatively short growing seasons.

Beneficial associations have been reported between total protein [216;495] and DF [216;305;570] intakes, and body weight and body composition for both children and adults. Diets rich in proteins and DF affect body weight and body composition by stimulating satiety and, therefore, promoting the control of energy intake, regulating the appetite and decreasing dietary intake [279]. An inverse association was found between plant protein intake, and body weight and central OB in recent cross-sectional studies [72;365]. On the other hand, a positive association was observed between animal protein intake and BMI in a recent German longitudinal study involving young adolescents [209]. Previous reports of the CHNS data (1989-2004) indicated that dietary protein intake among the urban and rural Chinese adults (18-45 years) were in line with the WHO recommendations [545;573], but not DF intake reported from CHNS data (1991-2011) (≥ 45 years) [518;551]. Moreover, an undesirable shift from carbohydrates to fat in the diet and a profound increase in BMI distribution and hypertension were observed [520;573;574]. The potential impact of dietary protein and DF intakes on OB has been evaluated via socio-economic status and demography (urban *vs.* rural) [519]. However, a comprehensive investigation of dietary protein and DF intakes among dietary culture based regions in China is still lacking. The rapid increased prevalence of Chinese OB has been proved as the result of the changes in dietary habits. To gain better knowledge of the association with OB, dietary protein and fiber from the detailed food groups may explain the potential risks and factors. Therefore, the present study aimed to compare total, animal, and plant protein, and DF intakes between gender-age and dietary region-age subgroups in the CHNS 2004 cross-sectional study and to investigate how food group-specific protein and fiber intakes relate to measurements of anthropometry (BMI and WC).

3. METHODS

3.1. Study population and design

The CHNS 2004 cross-sectional study involved Chinese of both genders from the nine provinces (Guangxi, Guizhou, Heilongjiang, Henan, Hubei, Hunan, Jiangsu, Liaoning, and Shandong). A multistage, random-cluster sampling procedure, taking into account demography, socioeconomic and health status, and nutritional characteristics, was used to draw representative samples from each of the nine provinces [577]. Four counties and two cities were randomly selected within each province. Neighborhoods were randomly selected from cities, suburbs, townships and villages. Data was collected at both household and individual levels. The inclusion criteria: 1) participants older than 3 years; 2) reporting the demographic information such as gender, age, province and urban/ rural; 3) living in the reported province (urban or rural) during the survey; 4) physically and mentally healthy; 5) participating in all the interviews with valid dietary intake. More details can be found elsewhere [557].

In the present study, participants (≥3 years) with three good-quality dietary interviews were included in the analyses. They were stratified in three gender-age groups: preschoolers (3-6 years), school-aged children (7-17 years), and adults (≥18 years). Moreover, as dietary culture and habits differ markedly across China, five regions with different types of diet [437], were defined: heavy meals (incl. energy-dense foods and oil) (HM) (e.g. Liaoning and Heilongjiang), sweet but less heavy meals (SM) (e.g. Jiangsu), hot and spicy meals (HS) (e.g. Hunan, Hubei and Guizhou), flour (pasta)-rich meals (FM) (e.g. Shandong and Henan), and variety meals (often with sour taste) (VM) (e.g. Guangxi).

The protocol of the survey was approved by the Ethical Committee of the National Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention. Signed informed consent forms were obtained from all participants, including parents or guardians and the children themselves.

3.2. Dietary intake assessment

Three consecutive 24-h dietary recalls were used to collect individual dietary intake data on all food consumed away from home and at home. Parents or guardians recalled the consumption of the young children (<12 years). The three consecutive 24-h dietary recalls were randomly allocated and almost equally balanced across the seven days of the week. Using food models and pictures, well-trained interviewers recorded the types and amounts of all food items consumed during the previous day together with the place of consumption [521].

The quality of the data collection was checked by comparing an individual's average daily dietary intake, calculated from the household survey, with the dietary intake data obtained via the 24-h dietary recalls, respectively. In case of significant discrepancies, the household participants and individual dietary data were re-contacted and rechecked, respectively.

Total protein, animal and plant protein, and water-insoluble fiber (WIF) intakes were estimated using the Chinese Food Composition Table (2002) [114]. Food group-specific protein intakes were calculated for meat, dairy, eggs, fish, cereals, potatoes, legumes, vegetables, fruits, and other sources. The amount of WIF for each food was available from the Chinese Food Composition Table, which was measured using the neutral detergent method approved and adapted by the AOAC [518]. Individual daily intake value of each food item was provided by the dietary data. Food group-specific total DF intakes were calculated for cereals, potatoes, legumes, vegetables, fruits, and other sources based on the food-group specific ratio of WIF/total DF [575]. Therefore, the calculation of total DF is based on the formula: total DF= 1.35 * cereal-derived DF + 1.74 * potato-derived DF + 1.68 * legume-derived DF +1.77 * vegetable-derived DF + 1.46 * fruit-derived DF + 1.61 * DF from other sources. Under-reporters and over-reporters, whose estimated energy intakes were higher than 5000 Kcal/d and lower than 500 kcal/d, were excluded [518].

3.3. Anthropometry

Weight (kg), height (cm), and WC (cm) were obtained during interviews, following standardized procedures [516].

BMI was calculated as weight (kg)/height^2 (m^2). Preschoolers [103] and school-aged children [191] were defined as OW and OB using age- and gender-specific BMI cut-off points between the 85th and 95th percentile, and at the 95th percentile or higher, respectively, as proposed by the International Obesity Task Force (IOTF) [103] and the Working Group for OB in China (2004) [191]. Adults with a BMI value of 24–28 kg/m^2 and ≥28 kg/m^2 were defined as OW and OB, respectively, as recommended by the Working Group for OB in China [107].

To define abdominal adiposity, the cut-off values of WC described by Ma *et al.* [317], were used for school-aged children. For adults, WC values were considered borderline when reaching 85-95 cm for men and 80-90 cm for women, and too large when exceeding 95 cm for men and 90 cm for women, as suggested by Chen *et al.* (2004) [96].

3.4. Statistical analysis

Descriptive statistics of the study population (mean values and standard deviations (SD)) were calculated by gender-age and dietary habit region-age groups. The statistical differences in body composition, energy, dietary protein and fiber intakes between the gender-age and dietary region-age strata were assessed using Bonferroni correction of UNIANOVA and MANCOVA. The dietary intakes of preschoolers were log-transformed. Results were considered significant at an α two-tailed level of 0.05.

A generalized linear model (GLM) was used to investigate the associations between food group-specific protein and DF intakes, and anthropometric data (BMI/BMI z-score and WC/WC z-score) after adjustment for gender, age, demography (province, urban or rural), dietary habit region, (parental) education (no study completed, primary school, lower middle school, upper middle school, technical or vocational education, master degree or higher, or student), (parental) occupational status (unemployed, employed, retired, or other), and energy intake (DF intake). Two-way interactions were tested between gender, age, demography, and dietary habit region, and food group-specific protein or DF intakes.

Statistical power for statistical analysis was calculated by PASS (2014) [359]. All statistical analyses were performed using SPSS for Windows version 20.0 (SPSS Inc Chicago, IL, USA).

4. RESULTS

11727 out of 12389 invited individuals willing to participate in this study were considered as qualified candidates. In total, 9720 individuals (≥3 years; 78.5% of 2004) provided three complete good-quality 24-h dietary recalls (Table 6.1.). The prevalence of individuals with an unhealthy body composition was the highest in the adult groups. The prevalence of borderline and abdominal adiposity was higher in female adults (28.3% *vs.* 31.2%, P = 0.001; 12.3% *vs.* 16.2, P = 0.007, respectively). Whereas, the prevalence of obese preschoolers was much higher among boys than girls (18.4% *vs.* 9%, P = 0.020). The lowest prevalence of OW and OB with healthy body composition was found in the VM region. Conversely, in the FM region, proportionally more persons with OW and OB and/or borderline or too large WC were found.

Table 6.1. Participants (n (%)) of the China Health and Nutrition Survey (2004) stratified in gender-age and dietary region-age groups (n= 9720)

Items	Age-group		
	3-6 years (n=328) §	**7-17 years (n=1334)**	**≥18 years (n=8058)**
		n (%)	
Gender			
Male	193 (58.8)	698 (52.3)	3845 (47.7)
Female	135 (41.2)	636 (47.7)	4213 (52.3)
Dietary culture regions $^{\mu}$			
Heavy meals (incl. energy-dense foods and oil)	47 (14.3)	285 (21.4)	1618 (20.1)
Sweet but less heavy meals	34 (10.4)	132 (9.9)	989 (12.3)
Hot and spicy meals	102 (31.1)	477 (35.8)	2498 (31.0)
Flour (pasta)-rich meals	85 (25.9)	253 (18.9)	1854 (23.0)
Variety meals	60 (18.3)	187 (14.0)	1099 (13.6)
Anthropometry			
Weight status as BMI categories (n=9043)			
Underweight	140 (51.1)	0 (0.0)	461 (6.1)
Normal-weight	63 (23.0)	1084 (89.3)	4350 (57.6)
Overweight	31 (11.3)	86 (7.1)	2120 (28.1)
Obesity	40 (14.6)	40 (3.6)	624 (8.3)
Overweight in dietary culture regions $^{\mu}$			
Heavy meals	4 (10)	23 (8.3)	510 (32.5)

Sweet but less heavy meals	2 (6.7)	12 (9.4)	306 (31.7)
Hot and spicy meals	61 (9.2)	25 (5.7)	542 (23.1)
Flour (pasta)-rich meals	11 (16.2)	21 (10.3)	576 (34.9)
Variety meals	6 (12.2)	5 (2.9)	186 (18.3)
Obesity in dietary culture regions‡			
Heavy meals	6 (15)	8 (2.9)	174 (11.1)
Sweet but less heavy meals	2 (6.7)	8 (6.3)	92 (9.5)
Hot and spicy meals	18 (20.7)	13 (3.0)	128 (5.4)
Flour (pasta)-rich meals	12 (17.6)	10 (4.9)	205 (12.4)
Variety meals	2 (4.1)	5 (2.9)	25 (2.5)
Waist circumference categories (n=9093)			
Borderline	NA	181 (14.8)	2250 (29.8)
Too large	NA	114 (9.3)	1082 (14.4)
Borderline WC in dietary culture regions$^{\mu}$			
Heavy meals	NA	50 (18.2)	510 (32.4)
Sweet but less heavy meals	NA	21 (16.7)	314 (32.5)
Hot and spicy meals	NA	52 (11.4)	669 (28.7)
Flour (pasta)-rich meals	NA	39 (18.7)	542 (32.8)
Variety meals	NA	19 (11.0)	215 (21.2)
Too large WC in dietary culture regions$^{\mu}$			
Heavy meals	NA	32 (11.6)	281 (17.9)
Sweet but less heavy meals	NA	14 (11.1)	157 (16.3)
Hot and spicy meals	NA	44 (9.7)	247 (10.6)
Flour (pasta)-rich meals	NA	26 (12.4)	343 (20.7)
Variety meals	NA	8 (4.7)	54 (5.3)
		Mean (SD)	
BMI (kg/m^2)			
Total group (n=9043)	15.9 (2.4)	18.0 (2.9)	23.1 (3.4)
Gender			
Male	15.8 (2.0)	18.1 (2.9)	23.0 (3.2)
Female	15.9 (2.9)	17.9 (2.9)	23.2 (3.5)**
BMI in Dietary culture regions$^{\mu}$			
Heavy meals	16.4 (2.7)	18.3 (2.9)	23.7 (3.5)
Sweet but less heavy meals	15.9 (2.9)	18.4 (3.2)	23.5 (3.4)
Hot and spicy meals	15.2 (1.4)	17.6 (2.8)	22.5 (3.2)
Flour (pasta)-rich meals	16.9 (3.3)	18.8 (2.8)	24.1 (3.4)
Variety meals	15.3 (1.9) †	17.3 (2.6) †	21.7 (2.9) †

Waist Circumference (cm)			
Total group (n=9093)			
Gender			
Male	51.4 (5.1)	74.9 (9.9)	82.7 (9.8)
Female	49.9 (7.5)	62.0 (8.7)*	79.5 (10.0*
WC in Dietary culture regions$^{\mu}$			
Heavy meals	50.6 (7.3)	65.2 (9.5)	82.6 (10.3)
Sweet but less heavy meals	53.7 (6.2)	64.0 (9.5)	82.0 (9.8)
Hot and spicy meals	49.2 (6.1)	62.5 (9.4)	79.7 (9.4)
Flour (pasta)-rich meals	52.1 (5.8)	65.2 (9.4)	83.5 (10.2)
Variety meals	49.6 (4.7) †	61.2 (8.8) †	76.7 (9.1) †

$^{\mu}$China was divided into 5 regions with different types of diet: heavy meals (incl. energy-dense foods and oil) (Liaoning and Heilongjiang), sweet but less heavy meals (Jiangsu), hot and spicy meals (Hunan, Hubei and Guizhou), flour (pasta)-rich meals (Shandong and Henan), and variety meals (often with sour taste) (Guangxi).

§ Mean BMI z-score and waist circumference z-score are for preschoolers. Mean BMI z-score: male, 0.281; female, -0.021; heavy meals, 0.302; sweet but less heavy meals, 0.090; hot and spicy meals, 0.219; flour (pasta)-rich meals, 0.450; variety meals, -0.426. Mean waist circumference z-score: male, -0.372; female, -0.948; heavy foods and oil, -0.791; light and sweet foods, 0.373; hot and spicy foods, -1.1; flour (pasta), -0.220; variety foods, -0.808.

* *P* value for mean differences between males and females, $P \leq 0.001$ (UNIANOVA, Games-Howell).

** *P* value for mean differences between males and females, $P < 0.05$ (UNIANOVA, Games-Howell).

† *P* for trend based on dietary culture categories, $P \leq 0.001$ (UNIANOVA).

‡ *P* for trend based on dietary culture categories, $P<0.05$ (UNIANOVA).

4.1. Protein and fiber intakes

School-aged boys and adult men had significantly higher intakes of energy, total protein, animal, and plant protein, and DF than school-aged girls and adult women (Table 6.2.). Whereas, energy-adjusted plant protein and DF intakes were significant higher in female adults than male adults. On the other hand, after adjustment for energy intake, statistical significance of DF disappeared in school-aged children. Total protein intake contributed 12.3% to the energy intake in all gender-age and dietary region-age strata (Table 6.2. and 6.3.). On average, 73.8% of the dietary proteins were from vegetable origin. Cereals were the main contributor to the total protein intake in all gender-age and dietary region-age strata, followed by meat, legumes and vegetables (data not shown). The food groups contributing most to the DF intakes were vegetables, followed by cereals and legumes (data not shown).

The mean intakes of energy, total and plant proteins, and proteins contributing to energy, and DF intakes differed significantly between the Chinese dietary regions-age strata (Table 6.3.). The highest energy, total and animal protein intakes were reported in SM (P < 0.05) and relatively lower in HM. Preschoolers and school-aged children in VM (P< 0.05) and FM had the lowest and highest DF intakes, respectively, whereas in the adult group, DF were the least consumed in VM (P<0.05 for energy-adjusted DF) and the most in SM (P <0.05). After adjustment for energy, total and animal protein were higher in SM, but lower in HM and FM, respectively. On the other hand, the energy-adjusted plant protein and DF intake were found highest in FM, adults in particular.

Table 6.2. Mean (SD) daily intakes of energy, proteins (total, plant, and animal), and fibers (total and insoluble) and the energy contribution of total proteins among Chinese participants stratified in gender-age and dietary region-age groups (n= 9720)

	Age Group		
	3-6 (n=328) †	**7-17 (n=1334)**	**≥18 (n=8058)**
Gender: male (n=4736)			
Energy (kcal/d)	1274.3 (604.9)	2100.4 (737.9)	2460.6 (741.4)
Total protein (g/d)	38.3 (18.7)	64.5 (26.0)	74.2 (26.2)
Total protein (g /(1000kcal*d))	30.7 (7.2)	31.1 (7.0)	30.5 (6.9)
Plant protein (g/d)	26.0 (14.0)	45.4 (19.1)	53.7 (21.3)
Plant protein (g/(1000kcal*d))	21.0 (6.1)	22.1 96.2)	22.1 (6.1)
Animal protein (g/d)	12.3 (12.6)	19.1 (18.0)	20.6 (18.2)
Animal protein (g/(1000kcal*d))	9.6 (7.9)	9.0 (7.8)	8.4 (6.9)
Energy contribution of total protein (%)	12.3 (2.9)	12.4 (2.8)	12.2 (2.7)
Energy contribution of plant protein (%)	8.4 (2.5)	8.8 (2.5)	8.8 (2.5)
Total fiber (g/d)	9.2 (6.8)	16.1 (12.9)	19.1 (15.5)
Fiber (g /(1000kcal*d))	7.5 (4.1)	7.9 (6.0)	7.8 (5.0)
Female (n=4983)			
Energy (kcal/d)	1289.5 (621.8)	1810.5 (607.5)*	2099.2 (680.6)*
Total protein (g/d)	40.3 (25.1)	55.8 (21.8)*	64.0 (23.4)*
Total protein (g/(1000kcal*d))	31.3 (6.0)	31.0 (7.2)	30.9 (7.1)**
Plant protein (g/d)	25.5 (11.2)	39.1 (15.9)*	46.4 (18.7)*
Plant protein (g/(1000kcal*d))	20.7 (6.5)	22.1 (6.6)	22.4 (6.1)**
Animal protein (g/d)	14.7 (21.8)**	16.7 (16.0)*	17.6 (16.0)*
Animal protein (g/(1000kcal*d))	10.5 (7.7)**	8.9 (7.7)	8.5 (7.1)
Energy contribution of total protein (%)	12.5 (2.4)	12.4 (2.8)	12.3 (2.8)**
Energy contribution of plant protein (%)	8.3 (2.6)	8.9 (2.6)	9.0 (2.4)**
Total fiber (g/d)	8.7 (5.1)	14.1 (9.4)**	17.2 (12.4)*
Fiber (g /(1000kcal*d))	7.2 (3.6)	8.1 (4.9)	8.3 (4.8)*

SD, standard deviation

† Dietary intakes of preschoolers were after log-transformation

*P value of intakes for mean differences between males and females, $P \leq 0.001$ (MANCOVA, Bonferroni correction).

**P value of intakes for mean differences between males and females, $P < 0.05$ (MANCOVA, Bonferroni correction).

Table 6.3. Mean (SD) daily intakes of energy, proteins (total, plant, and animal), and fibers (total and insoluble) and the energy contribution of total proteins among the China Health and Nutrition Survey (2004) participants stratified in dietary region-age groups $^{\mu}$ (n= 9720).

	Age Group		
Dietary region	**3-6† (n=328)**	**7-17 years (n=1334)**	**≥18 years (n=8058)**
Heavy meals (n=1950)			
Energy (kcal/d)	1195.6 (541.1)	1854.5 (671.0)^{b}	2100.5 (652.1)^{bcde}
Total protein (g/d)	34.5 (17.4)^{b}	54.0 (21.3)^{bc}	61.9 (23.8)^{bcd}
Total protein (g/(1000kcal*d))	28.6 (5.2)^{b}	29.3 (6.5)^{bc}	29.6 (7.0)^{bcd}
Plant protein (g/d)	22.0 (8.8)	39.3 (14.3)^{cde}	45.8 (17.4)^{bcde}
Plant protein (g/(1000kcal*d))	19.3 (4.9) ^{d}	21.8 (5.4)^{bcde}	22.1 (5.4)^{bcde}
Animal protein (g/d)	12.5 (12.8)^{b}	14.7 (15.1)^{be}	16.1 (15.8)^{bcde}
Animal protein (g/(1000kcal*d))	9.4 (7.0)^{b}	7.6 (7.3)^{bde}	7.5 (6.9)^{bcde}
Energy contribution of total protein (%)	11.5 (2.1)^{b}	11.7 (2.6)^{ab}	11.8 (2.8)^{bcd}
Energy contribution of plant protein (%)	7.7 (2.0)^{d}	8.7 (2.2)^{bde}	8.8 (2.2)^{bcde}
Total fiber (g/d)	8.2 (6.1)	14.1 (7.3)^{e}	17.6 (10.0)^{bde}
Fiber (g /(1000kcal*d))	6.9 (2.9)	8.2 (5.4)^{e}	8.7 (4.1)^{bde}
Sweet but less heavy meals (n=1155)			
Energy (kcal/d)	1585.2 (811.7)	2279.7 (801.9)^{acde}	2601.1 (801.3)^{acde}
Total protein (g/d)	52.5 (25.2)^{ae}	74.4 (28.2)^{acde}	80.2 (26.3)^{acde}
Total protein (g/(1000kcal*d))	34.3 (8.9)^{ae}	33.6 (8.8)^{ade}	31.5 (7.2)^{ade}
Plant protein (g/d)	29.1 (20.3)	42.5 (17.0)^{e}	53.1 (22.5)^{ae}
Plant protein (g/(1000kcal*d))	18.1 (6.1)^{cd}	18.9 (5.0)^{acd}	20.5 (5.6)^{acde}
Animal protein (g/d)	23.4 (18.3)^{acde}	31.9 (22.6)^{acde}	27.1 (17.2)^{acde}
Animal protein (g/(1000kcal*d))	16.2 (9.7)^{acd}	14.7 (9.3)^{acd}	11.0 (7.0)^{acd}
Energy contribution of total protein (%)	13.7 (3.6)^{ae}	13.4 (3.5)^{acde}	12.6 (2.9)^{ade}
Energy contribution of plant protein (%)	7.2 (2.4)^{cd}	7.6 (2.0)^{acd}	8.8 (2.2)^{acde}
Total fiber (g/d)	10.1 (7.9)	15.8 (8.1)^{e}	20.1 (12.1) ^{ae}
Fiber g /(1000kcal*d)	6.4 (3.3)	7.1 (3.4)	7.8 (3.9)^{ade}
Hot and spicy meals (n=3077)			
Energy (kcal/d)	1217.7 (660.2)	1981.5 (694.0)^{b}	2318.8 (715.5)^{abde}

Total protein (g/d)	39.2 (28.1)	62.1 (24.2)^{ab}	72.5 (25.4)^{abde}
Total protein (g/(1000kcal*d))	32.2 (7.1)^{e}	31.8 (7.2)^{a}	31.9 (7.9)^{ade}
Plant protein (g/d)	25.7 (11.3)	44.8 (18.8)^{ae}	52.1 (20.6)^{ade}
Plant protein (g/(1000kcal*d))	22.6 (7.0)^{be}	23.3 (7.1)^{abde}	23.0 (6.9)^{abde}
Animal protein (g/d)	13.5 (24.2)^{b}	17.3 (16.4)^{bd}	20.4 (18.5)^{abde}
Animal protein (g/(1000kcal*d))	9.6 (8.0)^{b}	8.5 (7.2)^{bde}	8.9 (7.6)^{abde}
Energy contribution of total protein (%)	12.9 (2.8)^{e}	12.7 (2.9)^{ab}	12.8 (3.2)^{ade}
Energy contribution of plant protein (%)	9.0 (2.8)^{be}	9.3 (2.8) ^{bde}	9.2 (2.8)^{abde}
Total fiber (g/d)	8.8 (4.6)	16.3 (14.0)^{e}	18.7 (19.0)^{de}
Fiber g /(1000kcal*d)	8.1 (4.2)^{e}	8.5 (7.0)^{e}	8.2 (6.5)^{de}
Flour (pasta)-rich meals (n=2192)			
Energy (kcal/d)	1260.6 (522.5)	1933.5 (688.3)^{b}	2222.0 (755.0)^{abce}
Total protein (g/d)	37.9 (14.8)^{b}	58.5 (25.1)^{b}	67.2 (25.7)^{abce}
Total protein (g/(1000kcal*d))	30.7 (5.4)	30.4 (6.0)^{b}	30.5 (5.7)^{abce}
Plant protein (g/d)	28.7 (13.6)	47.3 (21.1)^{ae}	54.1 (22.0)^{ace}
Plant protein (g/(1000kcal*d))	23.1 (5.9)^{abe}	24.8 (6.2)^{abce}	24.6 (5.8)^{abce}
Animal protein (g/d)	9.2 (8.5)^{b}	11.3 (12.1)^{bce}	13.1 (15.1)^{abce}
Animal protein (g/(1000kcal*d))	7.6 (6.3)^{b}	5.6 (5.5)^{abce}	5.9 (5.5)^{abce}
Energy contribution of total protein (%)	12.3 (2.2)	12.2 (2.4)^{b}	12.2 (2.3)^{abce}
Energy contribution of plant protein (%)	9.2 (2.3)^{abe}	9.9 (2.5)^{abce}	9.8 (2.3)^{abce}
Total fiber (g/d)	10.7 (7.5)^{e}	17.0 (12.6)^{e}	20.0 (12.0) ^{acd}
Fiber g /(1000kcal*d)	8.6 (4.4)^{e}	8.8 (3.9)^{e}	9.1 (4.0)^{abce}
Variety meals (n=1346)			
Energy (kcal/d)	1309.5 (519.6)	1891.9 (582.5) *^{b}	2204.0 (674.8)*^{abcd}
Total protein (g/d)	36.8 (14.1) **^{b}	58.0 (21.6)*^{b}	63.3 (20.3)*^{bcd}
Total protein (g/(1000kcal*d))	29.0 (6.6)*^{bc}	30.9 (7.1) *^{b}	29.2 (6.0)*^{bcd}
Plant protein (g/d)	23.1 (10.6)**	34.4 (12.6)*^{abcd}	40.6 (13.5)*^{abcd}
Plant protein (g/(1000kcal*d))	17.9 (4.3)*^{cd}	18.2 (3.2)*^{acd}	18.6 (3.4)*^{abcd}
Animal protein (g/d)	13.7 (9.4)**^{b}	23.6 (16.0)*^{abcd}	22.7 (14.4)*^{abcd}
Animal protein (g/(1000kcal*d))	11.1 (7.1)*	12.6 (7.6)*^{acd}	10.6 (6.2) ^{acd}
Energy contribution of total protein (%)	11.6 (2.6)*^{bc}	12.3 (2.8)*^{b}	11.7 (2.4)*^{bcd}

Energy contribution of plant protein (%)	7.2 (1.7)* [cd]	7.3 (1.3)*[acd]	7.4 (1.4)*[abcd]
Total fiber (g/d)	7.0 (4.7)*[d]	10.7 (7.3)*[abcd]	12.3 (7.2)* [abcd]
Fiber g /(1000kcal*d)	5.4 (2.4)*[cd]	5.7 (2.9)*[acd]	5.6 (2.4)*[abcd]

SD, standard deviation

[µ] China was divided into 5 regions with different types of diet: heavy meals (incl. energy-dense foods and oil) (Liaoning and Heilongjiang), sweet but less heavy meals (Jiangsu), hot and spicy foods (Hunan, Hubei and Guizhou), flour (pasta)-rich meals (Shandong and Henan), and variety meals (often with sour taste) (Guangxi).

† Dietary intakes of preschoolers were after log-transformation

[*] *P* for trend based on dietary culture categories, $P \leq 0.001$ (MANCOVA).

[**] *P* for trend based on dietary culture categories, $P \leq 0.05$ (MANCOVA).

[a] *P* value for mean differences from the region of heavy meals, $P < 0.05$ (MANCOVA, Bonferroni correction).

[b] *P* value for mean differences from the region of light foods with sweet taste, $P < 0.05$ (MANCOVA, Bonferroni correction).

[c] *P* value for mean differences from the region of hot and spicy foods, $P < 0.05$ (MANCOVA, Bonferroni correction).

[d] *P* value for mean differences from the region of flour (pasta), $P < 0.05$ (MANCOVA, Bonferroni correction).

[e] *P* value for mean differences from the region of variety foods enjoying sour taste, $P < 0.05$ (MANCOVA, Bonferroni correction).

4.2. Associations between food group-specific protein and fiber intakes, and body composition

Higher BMIs were observed in preschoolers with low intakes of energy-adjusted total and animal protein, while higher BMIs were observed in school-aged children with lower intakes of energy-adjusted plant protein, DF and energy-adjusted DF and higher intakes of energy-adjusted animal protein, although no statistical significance was observed among preschoolers and school-aged children (Figure 6.1.). School-aged children with higher energy-adjusted total and animal proteins intakes had too large WC values (Figure 6.2.). However, after adjustment for energy, DF decreased slightly with increasing WC. For the adults, higher intakes of energy-adjusted total protein, animal and plant protein were found in OW subjects (energy-adjusted total protein: P<0.001) and higher intakes of energy-adjusted total and animal proteins were found in subjects with borderline WC (energy-adjusted total protein: P <0.001). While, energy-adjusted plant protein and energy-adjusted fiber were consumed most in the subjects with too large WC (plant protein: P<0.001).

Associations between food group-specific protein and fiber intakes, on one hand, and BMI and WC (Table 6.4.), on the other hand, were investigated by GLM. Significant positive associations were found between the meat- and fish-derived protein intakes of school-aged children and their BMI, and between their dairy- and potato-derived protein intakes and WC. In adults, both BMI and WC were positively related with the protein intakes through the consumption of fish and legumes. Moreover, in this age-group, inverse associations were found between some food group-specific protein intakes and BMI (dairy and potatoes), and WC (dairy, vegetables, and fruits). Only fish-derived protein was inversely associated with preschoolers' WC (B= -0.092, P= 0.033).

Furthermore, the intake of cereal-derived fiber was positively associated with the BMI and WC of preschoolers. However, an inverse relation was observed between cereal-derived fiber intakes and the BMI of school-aged children, and both the BMI and WC of adults. The intake of DF derived from legumes, however, was inversely associated with the WC of school-aged children, but positively with the BMI and WC of adults. Likewise, opposite associations were observed between the intake of fruit-derived fiber and the WC of school-aged children (B= 1.6, P= 0.015) and of adults (B= -0.388, P= 0.049). In the latter group, an inverse relation was found between vegetable-derived fiber intake and body composition.

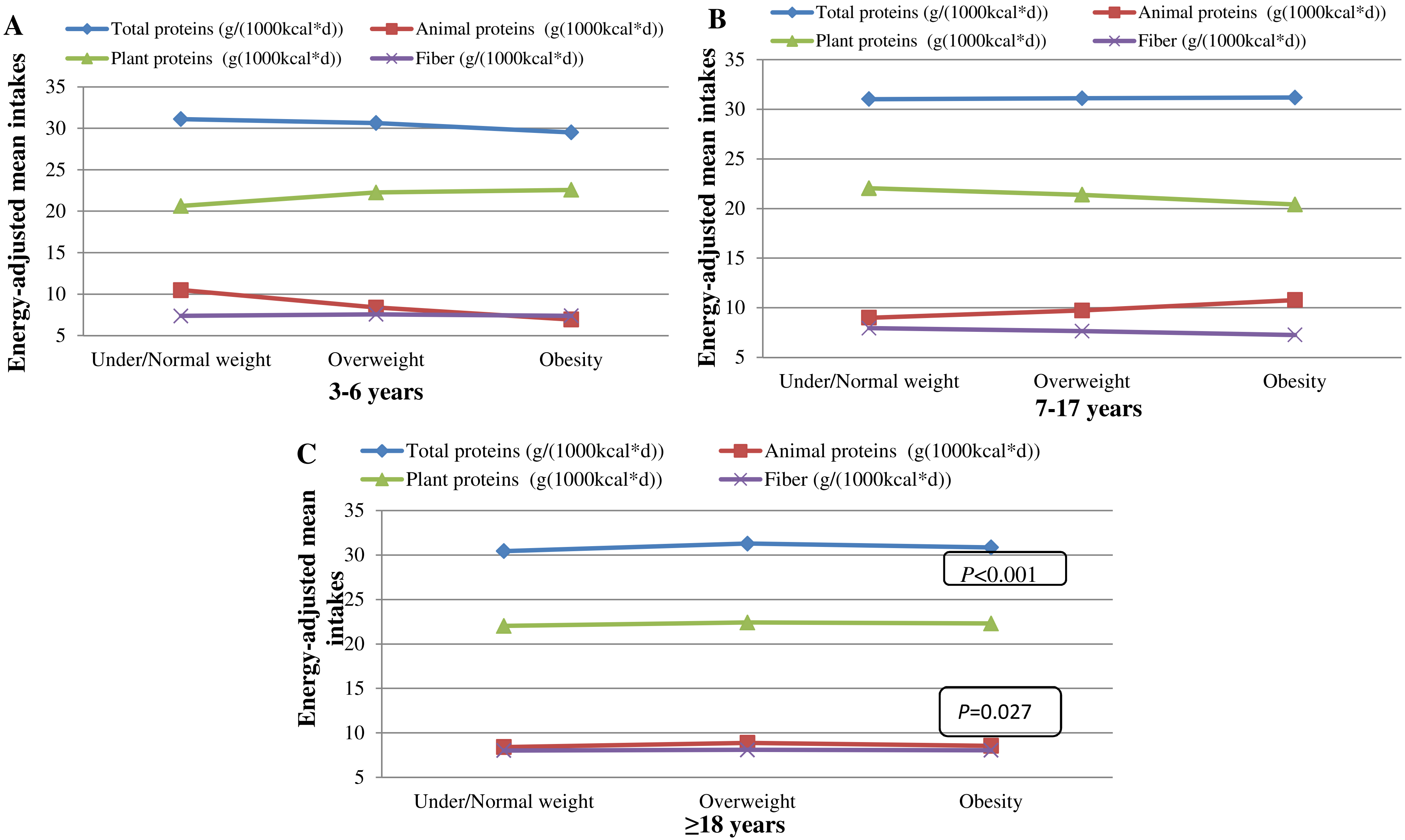

FIGURE 6.1. Energy-adjusted mean intakes based on BMI categories stratified by age groups (preschoolers (3-6 years): A; school-aged children (7-17 years): B; adults (≥18 years): C)

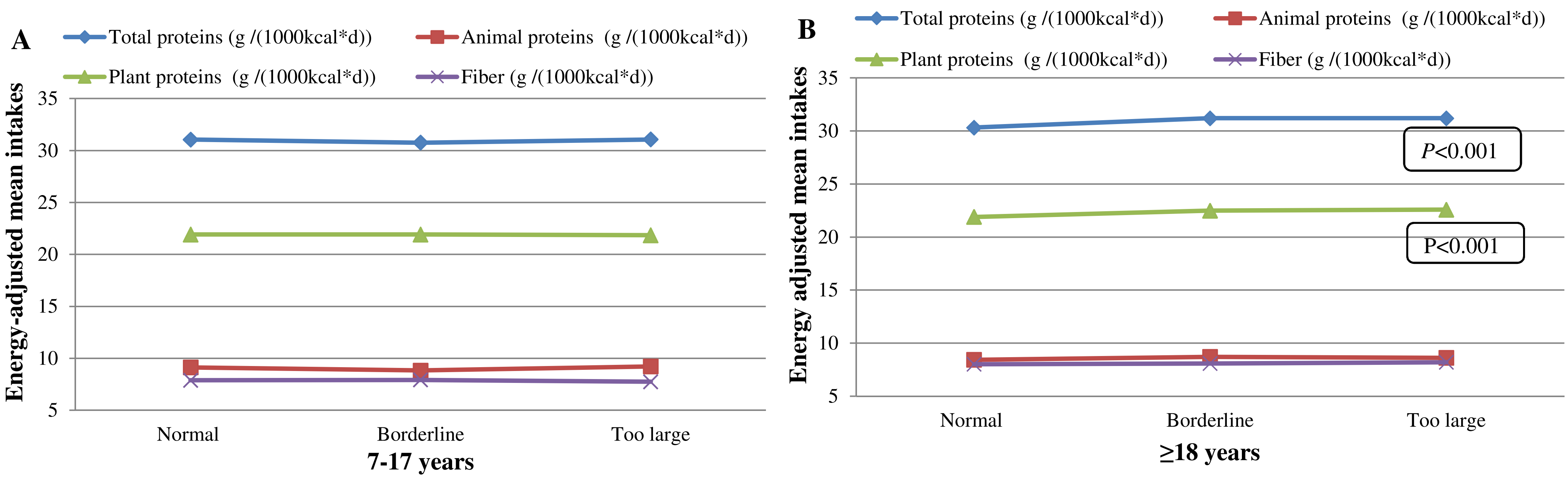

FIGURE 6.2. Energy-adjusted mean intakes based on waist circumference categories stratified by age groups (school-aged children (7-17 years): A; adults (≥18 years): B)

Table 6.4. Generalized linear model analyses of the potential association between food group-specific protein and fiber intakes and anthropometry for the Chinese population participating in the China Health and Nutrition Survey (2004) (n= 9043)

Food group	Protein intake											
	BMI z-score /BMI						**WC/WC z-score**					
	Preschoolers		**School-aged children**		**Adults**		**Preschoolers**		**School-aged children**		**Adults**	
	B **(SE)**	***P***	B **(SE)**	***P***	B **(SE)**	***P***	B **(SE)**	***P***	B **(SE)**	***P***	B **(SE)**	***P***
Meat	-0.048 (0.026)	0.064	0.015 (0.007)	0.038	-0.014 (0.011)	0.211	0.007 (0.018)	0.697	0.025 (0.018)	0.171	0.041 (0.025)	0.102
Dairy	0.005 (0.025)	0.838	-0.022 (0.090)	0.807	-0.070 (0.019)	<0.001	0.026 (0.040)	0.509	0.353 (0.176)	0.045	-0.235 (0.055)	<0.001
Eggs	-0.007 (0.006)	0.283	-0.026 (0.063)	0.680	0.001 (0.007)	0.942	-0.001 (0.011)	0.897	-0.117 (0.081)	0.149	-0.027 (0.021)	0.193
Fish	-0.073 (0.058)	0.210	0.104 (0.048)	0.030	0.023 (0.006)	<0.001	-0.092 (0.043)	0.033	0.038 (0.040)	0.340	0.079 (0.017)	<0.001
Cereals	0.017 (0.010)	0.081	-0.036 (0.025)	0.145	-0.002 (0.003)	0.629	0.016 (0.015)	0.290	0.028 (0.020)	0.165	0.017 (0.028)	0.548
Potatoes	0.016 (0.150)	0.913	0.042 (0.083)	0.611	-0.244 (0.098)	0.013	0.385 (0.233)	0.099	0.589 (0.226)	0.009	-0.114 (0.091)	0.211
Legumes	0.001 (0.016)	0.949	-0.014 (0.008)	0.079	0.008 (0.003)	0.014	0.017 (0.024)	0.478	-0.037 (0.023)	0.105	0.033 (0.010)	0.001
Vegetables	-0.046 (0.051)	0.363	-0.014 (0.027)	0.590	-0.052 (0.032)	0.102	-0.097 (0.077)	0.210	0.080 (0.070)	0.252	-0.184 (0.094)	0.049
Fruits	0.275 (0.439)	0.532	-0.231 (0.262)	0.378	-0.822 (0.431)	0.057	0.511 (0.461)	0.268	3.609 (2.088)	0.084	-2.940 (1.254)	0.019
	Fiber intake											
Cereals	0.403 (0.170)	0.018	-0.327 (0.129)	0.011	-0.147 (0.053)	0.005	0.113 (0.044)	0.010	0.108 (0.061)	0.075	-0.146 (0.052)	0.005
Potatoes	0.208 (0.194)	0.281	0.052 (0.126)	0.682	-0.021 (0.040)	0.602	0.220 (0.296)	0.456	0.476 (0.338)	0.160	-0.027 (0.039)	0.495
Legumes	-0.004 (0.031)	0.903	-0.017 (0.015)	0.253	0.066 (0.025)	0.009	0.023 (0.049)	0.640	-0.093 (0.041)	0.023	0.067 (0.025)	0.008
Vegetables	-0.009 (0.033)	0.787	-0.018 (0.014)	0.214	-0.040 (0.017)	0.021	-0.030 (0.052)	0.570	-0.128 (0.080)	0.111	-0.041 (0.017)	0.018
Fruits	-0.042 (0.077)	0.584	-0.091 (0.066)	0.167	-0.104 (0.105)	0.324	0.167 (0.124)	0.179	1.580 (0.650)	0.015	-0.388 (0.197)	0.049

SE, standard error of coefficient B; CI, Confidence Interval.

5. DISCUSSION

To the best of our knowledge, these are few analyses on dietary protein and fiber intake in relation to OW and OB in Chinese regions with different types of diets. Although CHNS was not nationally representative, the diet patterns and anthropometry were similar to previous findings based on national cross-sectional surveys [181;398].

5.1. Dietary protein and fiber intakes

Mean the total protein and DF intakes of all participants in this large Chinese population-based national nutrition survey covering 9 provinces, were below the Chinese dietary intake guidelines [98], except for the total protein intake of inhabitants of SM. With regard to age groups, only one preschool boy and three school-aged boys met the recommended total protein intake by the Chinese dietary intake guidelines [98]. 33% and 21% of the adults met the recommendation based on the evaluation of moderate physical activity and heavy physical activity, respectively. In addition, only 9% adults met the recommended DF intake. However, in the whole study population, the energy contribution of total proteins was in line with the WHO/FAO/United Nations University recommendation (*i.e.*, 10–15% of the total energy intake) [545].

With China's rapid economic growth, Chinese dietary intakes and dietary behaviors changed gradually [66;573;574]. Although Chinese are undergoing a remarkably fast, but undesirable, shift towards a stage of nutrition dominated by a high intake of fat and animal foods, our results show that cereals were the main contributors to the total protein intake in all gender-age and dietary region-age strata. On average, 73.8% of the dietary proteins were from vegetable origin whereas in Western countries, the ratio of animal-to-plant protein intake approximates 3:1 [452;454]. Inhabitants of FM reported the lowest animal protein and energy-adjusted animal protein, and highest total and energy-adjusted fiber intakes, which is in line with their high consumption of flour-processed foods and relatively less varied diet. In SM, conversely, the highest animal protein and energy-adjusted animal protein consumption were observed, as well as the highest energy and total protein intakes due to the economic factor with relatively higher prevalence of OW and OB. The diet in this region is characterized by light and sweet foods and provided sufficient proteins to reach the Chinese dietary guidelines. Mean energy and protein intakes in the SM region were close to or exceeded the intakes as in Western populations [33;169]. In addition, DF intake in this rich region was among the highest. However, after adjustment for energy, DF intake turned out to be relatively lower in SM due to overconsumption. The inhabitants of HM consumed significantly the lowest energy intake and a relatively lower energy-adjusted animal protein intake, adult group in particular, due to the majority of the participants living in the rural areas with lower living standard and lower SES, although the dietary habits in the HM diet are rich in energy, saturated fatty acids and cholesterol. In addition, the inhabitants of HF consumed more stewed foods with a high intake of fat, oil and variety of plant foods, pickles in particular. Interestingly, no significantly low energy intake and energy-adjusted animal protein intake were found in the group of children, preschoolers and school-age children. Children would have got more attentions due to China's One Child Policy.

These findings suggest that, despite the nutrition transition, Chinese still have a mainly plant-based diet. In this present study, rural populations represented 66.7%, which may explain this high consumption of plant-based foods as the Western food transition in China mainly occurs in rich urban areas. But in SM, the dietary pattern has shifted towards an increasing consumption from animal sources. Animal protein contributed almost 50% to the total protein intake, which was closed to the Western dietary pattern [43]. Yet, the DF estimates were inadequate and comparable to Europeans in all age groups [99;150]. A possible explanation why the DF requirements are not met despite their plant-based diet could be because of the typical small portion sizes within this Chinese culture. Refined plant-based foods such as flour-processed foods in Northern China (e.g. FM region), are the main foods in the daily life, while grains, like rice, are main foods in the east and south regions (e.g. SM, VM). In addition, hot meals of Chinese eating habits may result in losing huge vitamins and DF during food preparation due to over-preparation.

5.2. Associations between protein and fiber intakes and body composition

The lowest prevalence of OW and OB with an unhealthy body composition was observed in the VM region, where the diet is varied and the plant protein and DF estimates amongst the lowest. OB and central adiposity were quite prevalent in the FM, HM, and SM regions. A large difference in the intake of wheat flour, associated with OB is energy density and cooking method. Compared to rice, wheat flour-processed foods such as noodles and steamed bread, which are more frequently consumed in FM, absorb less water during cooking and are, therefore, more energy-dense [565]. In addition, deep-fried flour-processed foods, deep-fried noodles and instant noodles are consumed regularly in FM, which are associated with OB because of the fat content, especially saturated fats and trans fats [43;292]. In contrast, participants from SM had the highest energy, total protein and animal protein intakes. Possible contributing factors to the over-nutrition in this region may be the economic prosperity resulting in a higher consumption of energy-dense, fat, and/or animal products, and the higher intake of sucrose through sugar consumption [13]. Interestingly, persons with an unhealthy body composition were less prevalent in the HS region where frying and seasoning are popular ways of preparing food. A possible explanation may be the control of postprandial hyperinsulinemia and adjustment of the lipid metabolism by hot chili due to increasing energy expenditure and fat oxidation [5].

In this present study, obese school-aged children and school-aged children with large WC consumed relatively higher energy-adjusted total and animal protein intakes and lower plant protein and fiber intakes. These findings among school-aged children are in line with a previous study conducted on adolescents [209]. In the adult group, OW adults consumed the highest intakes of energy-adjusted total protein, and animal and plant protein. But the highest intake of energy-adjusted total and plant protein, and energy-adjusted fiber were observed among adults with too large WC. These adults had a high consumption of dietary intakes. The potential reason can be that a proportion of female adults had a decreased protein intake with an increasing carbohydrate intake [143].

Furthermore, food-group specific protein and fiber were investigated the association of OB (BMI and WC) by GLM. Our findings, based on this large-population based study, reveals that some food-group specific plant-derived protein (potato, vegetables and fruits) intakes and fiber (cereals, vegetables and fruits) intakes were inversely associated with the BMI and/or WC of Chinese adults, which is consistent with previous reports [72;174;383]. Plant-derived proteins supply less fat or cholesterol, while DF-rich foods have a low glycemic index, which improve blood glucose level and lipid profiles, and support weight loss [175]. A population-based prospective study involving 64191 healthy middle-aged women in Shanghai, reported an inverse association between the consumption of vegetables but not of fruits, and the risk of T2D [510]. Fruit-derived fiber was positively associated with school-aged children's WC, which is supported by a recent study conducted on the Belgian population (≥ 15 years) [305]. A recent report derived from prospective cohort studies shows the weakly protective effects of vegetables and fruits are against OB and chronic diseases [450]. Similarly, our results show that potato-derived protein and cereal-derived fiber were positively associated with school-aged children's WC and preschooler's WC z-score, respectively, but inversely associated with school-aged children's WC. Most observational studies suggested that increasing the consumption of cereals and cereal products can benefit on weight loss and better WC, which can reduce the prevalence of OW and OB in children and adults [271;372;382]. In addition, a previous cross-sectional study supports our findings that potato consumption was positively associated with BMI and WC among Iranian adolescent girls due to high glycemic index [204]. No strong evidence can explain on the consumption of potato. Whereas, Chinese children were reported to consume potato chips and energy-dense snacks often out of home [297], which is associated with increased risk of OW and OB. However, frequent consumption of potato in China is low as it is prepared like vegetables instead of main foods. Steaming and boiling cooking ways are popular to prepare instead of deep-fried, which can explain the inverse association of adults.

Unexpectedly, in contrast to previous studies [163;265], dietary protein and DF intakes through the consumption of legumes and fish were positively associated with BMI and WC of adults participating in this present study. Conversely, legume-derived DF intake was inversely associated with WC of school-aged children. Soymilk, by far more popular than cow's milk in China, is a rich source of soy proteins, which effect insulin resistance, fatty acid metabolism lipid metabolism [499] by decreasing total, VLDL, and LDL plasma cholesterol and increasing HDL cholesterol [70] due to impact on satiety. A longitudinal study showed that legume consumption benefited to a better body weight and WC and lower SBP among legume consumers compared to non-consumers [384]. A cross-sectional study conducted on Iranian adolescents, by Kelishadi *et al*., found no association of soy consumption with BMI [262]. Legumes were the main contributors to Chinese daily intakes. Insufficient evidence of the observational studies on the relation between legumes and body weight and body composition was found in both children and adults literature. The potential reason can be too much legume consumptions resulting in high energy intake. Kim *et al.* reported that the grains, vegetables, and fish pattern was associated with a 14% lower risk of metabolic syndrome, especially lower risk of hypertriglyceridemia in Korean adults [265]. Yet, after a fish protein meal, the energy intake of normal-weight men (20-32 years) was 11% lower than following a beef protein meal [69]. However, some suggest that animal protein intakes might be positively associated with OB [303], which supports our positive association of meat-derived protein with BMI among school-aged children. No association of meat-derived protein was observed among adults, perhaps because the amount of meat consumption was limited and white and red meat were classified into the same group of meat in this present study. Red meat-derived protein was reported to be positively associated with body weight and WC among adults [59;101;344]. On the other hand, white meat-derived protein including poultry or fish, was not associated with OB [101]. One recent prospective study conducted on over half a million people reported that white meat-derived protein can protect CVD and the consequence of CVD [444].

Interestingly, dairy-derived protein intake was also inversely associated with the body composition of Chinese adults, which may be due to the effect of dairy-derived proteins on food intake, satiety, and short-term regulatory mechanisms related to usual serving portion sizes [316]. But dairy-derived protein was weakly positively associated with school-aged children's WC in this present study. The consumption of milk and milk products in China is not popular due to many factors such as acceptance of Western food items and lactose intolerance in majority of the Chinese population. Previous observational studies suggested dairy product consumption was positively associated with body weight [163;405]. A 12-year prospective cohort study indicated that increasing dairy-derived protein impacted on slightly weight gain among male adults [405]. On the contrary, dairy-derived protein from fermented dairy products was inversely associate with OB status among Brazilian adults [137]. In addition, in a randomized trial with OW or obese women, higher total protein and dairy-derived protein intakes promoted a more favorable body composition by total and visceral fat loss and lean mass gain during a diet- and exercise-induced weight loss [256].

Moreover, some unexpected positive associations were found in our study, including the intakes of meat- and fish-derived proteins, dairy-derived proteins, cereal-derived fiber with the BMI and WC of preschoolers or school-aged children. One recent cross-sectional study revealed the changes of dietary habits among Chinese children such as such as eating breakfast outside home, increased consumption of soft drinks, frequent consumption of energy-dense food, snacking and consuming preserved fruits, sweets and chocolates [297] due to economic growth. Chinese children got over feeding due to special Chinese policy— China's One Child Policy. In addition, the pre-puberty and puberty may have different effect on the school-aged children, adolescents in particular [97].

5.3. Strengths and limitations

The present data was collected from CHNS, which is the largest Chinese survey covering 9 provinces and taking into account differences in demography. In the present study, BMI and WC were used to investigate the associations between dietary protein and DF intakes and body composition associations.

Several limitations of this study should be noted. Firstly, because of the cross-sectional design of CHNS data, causality cannot be drawn. In addition, data obtained from 24-h dietary recalls may not necessarily reflect typical consumption patterns and infrequently consumed foods. However, 24-h recalls used in epidemiologic studies have been shown to produce reasonably accurate group estimates of nutrient intakes in large population-based studies. Because children's (<12 years) dietary intakes were reported by parents/proxies, reporting errors may have occurred and accuracy may be biased, for preschoolers in particular. Even the information reported by adults might be imprecise as the method relies on the participants' memory. Also food composition data might introduce some bias, though the accuracy of the Chinese food composition data used is unknown. Furthermore, the sample size of the preschoolers' group was very small compared to school-aged children and adults. However, the strong statistical power proved valid results. Finally, this study did not include information on physical activity, which may affect the relation between dietary intake and BMI and body composition.

6. CONCLUSION

Our results indicate that a plant-based diet prevailed in some participating regions of China. On average, 73.8% of the dietary proteins were from vegetable origin. Yet, mean total protein and DF intakes were below the Chinese dietary intake guidelines and differed significantly between the Chinese dietary culture regions-age strata. Relatively unhealthy body composition was observed in the SM region with the highest total and animal protein intakes. In adults, dairy protein intake and the intakes of protein and DF from cereals and vegetables were inversely associated with both BMI and WC, whereas the intakes of proteins and DF through the consumption of fish and legumes were positively related to these anthropometric measurements. Few associations were found among preschoolers and school-aged children.

CHAPTER 7

DISCUSSION

1. SUMMARY OF MAIN FINDINGS

1.1. Dietary protein and fiber intakes from the four research surveys

1.1.1. Dietary total, animal and plant protein intakes

Dietary total, animal and plant protein intakes in this thesis were evaluated in four regions or countries, including the Belgian population (≥ 15 years), preschoolers in Flanders (2.5-6.5 years), European adolescents (12.5-17.5 years) and the Chinese population (≥ 3 years). Total dietary protein intake in all the target populations reached the recommendations proposed by WHO/FAO/United Nations (i.e. 10–15% of the total energy intake) [545]. Because of the specific age group and country, dietary total protein intake in Flemish preschoolers, European adolescents and the Chinese population were evaluated by BSHC [160], EFSA [157], and the Chinese dietary intake guidelines [98], respectively, which states Flemish preschoolers and European adolescents were in line with the RDA [160] and PRI [157]. While, protein intake in the Chinese population shows that total protein intake was inadequate based on the Chinese guidelines, except for the total protein intake of inhabitants of SM, although total protein intake met the international recommendation mentioned above.

Considering the contribution of food sources to total protein intake, animal protein contributed approximately 2/3 (67%) to the total protein intake in the target European populations (Belgian population (64%), Flemish preschoolers (69%) and European adolescents (59%), which was the opposite to the results found in the target Chinese population (26%) (Table 7.2.). *Chapter 6, Appendix 13 and Appendix 14* show that the ratio of animal and plant protein intakes was 2/5 in the target Chinese population (*Appendix 14*). This is the first study to investigate dietary protein intakes and dietary habit regions in the Chinese population. The findings based on the results of the China study reveal that energy, total, animal and plant protein intakes were much higher in the SM region, while energy and total protein intakes were reported the lowest in the HM region.

One of the main findings is that meat and meat products were the main contributors to total and animal protein intakes for all the target populations with the exception for the Chinese population, whereas cereals and cereal products contributed most to plant protein intake in the target population [303;304] (Table 7.1.). Meat and meat products, and cereals and cereal products were the food sources contributing most to animal protein and plant protein, respectively, among Flemish preschoolers, the Belgian population, and European adolescents. In addition, the results from all the research studies state that protein from high nutritious and low energy-dense food sources such as fish and shellfish, vegetables, fruits and legumes were consumed in a low amount. On the other hand, energy-dense and low nutritious foods were a main contributor to Flemish preschoolers and European adolescents.

Table 7.1. The top five contributors to total protein intake in the target study populations

Ranking	Belgian population	Flemish preschoolers	European adolescents	Chinese population		
				Preschoolers	School-aged children	Adults
1	Meat and meat products	Meat, poultry, fish, and eggs	Meat, poultry, fish, eggs, nuts	Cereals and cereal products (including grains, flour)		
2	Cereals and cereal products (including bread, grains, flour)	Milk and milk products	Milk, milk products and cheese	Meat and meat products		
3	Dairy products	Bread and cereals	Bread and cereals	Legumes		
4	Fish and shellfish	Energy-dense and low nutritious foods	Potatoes and grains (mainly from pasta)	Vegetables		
5	Potatoes and other tubers	Cheese	Energy-dense and low nutritious foods	Egg	Fish	

1.1.2. Dietary fiber intakes

DF intake was inadequate in all four independent populations and below the recommendations proposed by BSHC [160], WHO [546], IOM [234], USDA [491], and the Chinese dietary intake guidelines [98] *(Chapter 3.3.2., Chapter 4.4.2., Chapter 5.5.2. and Chapter 6)*.

Regarding the food sources, cereals and cereal products were the largest contributor to DF intake in all the target populations with the exception of Chinese school-aged children and adults, who consumed mostly vegetables (*Chapter 3.3.2., Chapter 4.4.2., Chapter 5.5.2., Chapter 6 and Appendix 15*) (Table 7.2.). Similar as the findings on protein sources, legume-derived DF contributed only infinitesimally to the whole total DF intake in the Belgian population. In addition, SES (the level of education) was not an important factor of DF intake as presented in *Chapter 4.4.2.*, which states that men in the lowest education group and women with a higher education level had the highest mean DF intakes. WIF contributed more to DF intake than WSF in European adolescents and the Chinese population (*Chapter 5.5.2., Appendix 11, Appendix 12 and Appendix 13*). Considering the Chinese dietary habit regions, the finding of the Chinese study reveals that participants in the regions of VM and FM had the lowest and highest DF intakes, respectively.

Table 7.2. The top five contributors from food sources to total fiber intake in the target study populations

<table>
<tr><th rowspan="2">Ranking</th><th rowspan="2">Belgian population</th><th rowspan="2">Flemish preschoolers</th><th rowspan="2">European adolescents</th><th colspan="3">Chinese population</th></tr>
<tr><th>Preschoolers</th><th>School-aged children</th><th>Adults</th></tr>
<tr><td>1</td><td>Cereals and cereal products (including bread, grains, flour)</td><td>Bread and cereals</td><td>Bread and breakfast cereals</td><td>Cereals and cereal products (including grains, flour)</td><td colspan="2">Vegetables</td></tr>
<tr><td>2</td><td>Potatoes and other tubers</td><td>Fruits</td><td>Potatoes and grains</td><td>Vegetables</td><td colspan="2">Cereals and cereal products (including grains, flour)</td></tr>
<tr><td>3</td><td>Fruits</td><td>Potatoes and grains</td><td>Energy-dense and low-nutritious foods</td><td colspan="3">Legumes</td></tr>
<tr><td>4</td><td>Vegetables</td><td>Vegetables</td><td>Fruits</td><td colspan="3">Others (e.g. nuts, seeds)</td></tr>
<tr><td>5</td><td>Legume</td><td>Energy-dense and low-nutritious foods</td><td>vegetables</td><td>Fruits</td><td colspan="2">Potatoes</td></tr>
</table>

1.2. The associations of dietary protein and fiber intakes with obesity, and obesity related chronic diseases

1.2.1. The association between animal and plant protein intakes and overweight, obesity and cardio-metabolic indicators

Studies on the associations of OW and OB were investigated in the Belgian population, European adolescents and the Chinese population. The findings based on the results in this thesis show, on one hand, inverse associations of plant protein with BMI/BMI z-score and body composition of the Belgian population and European adolescents (Table 7.3.), on the other hand, positive associations of animal protein/ energy percentage of animal protein were found with BMI/BMI z-score and body composition of the Belgian population and European adolescents (*Chapter 4.4.1., Chapter 5.5.1.)*. Results of the Belgian population and European adolescents indicate that plant protein intake was inversely associated with body composition, which might assist in preventing against chronic diseases in the future. Few associations of animal and plant protein intakes were found with biomarkers. Only serum HDL-C was found to be weakly and positively associated with absolute animal protein intake in European adolescents. In the Chinese population, dairy-, potato-, vegetable- and fruit-derived protein intakes were inversely associated with BMI / WC of Chinese adults *(Chapter 6*), but positively with fish- and legume- derived protein intakes. In addition, potato- and dairy-derived protein intakes were positively associated with WC of school-aged children, besides the positive associations of BMI with meat- and fish- derived protein intakes. Only one inverse association was found between fish-derived protein intakes and preschoolers' WC.

Table 7.3. An overview of significant associations of animal and plant protein (g/d and E%) among the Belgian population and European adolescents, and protein from main food groups (g/d) among the Chinese population with obesity presented in the thesis

Food items	Belgian population				European adolescents †			
	BMI		Waist circumference		BMI z-score		Body fat (%)	
	B	*P*	B	*P*	B	*P*	B	*P*
Animal protein	0.013	0.001	0.041	0.002	NS	NS	–0.000052	0.004
Animal protein (E%)	NA	NA	NA	NA	0.024	0.010	0.168	0.017
Plant protein	-0.036	<0.001	-0.137	<0.001	–0.012	0.027	–0.139	0.001
Plant protein (E%)	NA		NA		NS		–0.229	0.130

Food groups	Chinese population‡							
	BMI				Waist circumference			
	School-aged children		Adults		School-aged children		Adults	
	B	*P*	B	*P*	B	*P*	B	*P*
Meat	0.015	0.038	NS		NS		NS	
Dairy	NS		-0.070	<0.001	0.353	0.045	-0.235	<0.001
Eggs	NS		NS		NS		NS	
Fish	0.104	0.030	0.023	<0.001	NS		0.079	<0.001
Cereals	NS		NS		NS		NS	
Potatoes	NS		-0.244	0.013	0.589	0.009	NS	
Legumes	NS		0.008	0.014	NS		0.033	0.001
Vegetables	NS		NS		NS		-0.184	0.049
Fruits	NS		NS		NS		-2.940	0.019

NA: not available, NS: not significant

†Only serum HDL-C was weakly associated with animal protein intake (B =0.001, *P*=0.039)

‡Chinese preschoolers was only associated with fish protein (B =-0.092, *P*=0.033)

1.2.2. The association between dietary fiber intakes and overweight, obesity and cardio-metabolic indicators

In *Chapter 4.4.2., Chapter 5.5.2. and Chapter 6*, the associations of the intakes of DF and the contributors to total DF with anthropometry and serum biomarkers have been described. The findings reveal inverse associations of total DF intake with BMI and body composition in the Belgian population. For example, increasing 1 g intake of total DF resulted in a reduction of 0.118 cm WC of the entire Belgian population. In addition, two types of DF (WSF and WIF) were further investigated in the European adolescents. The findings show that energy-adjusted WSF and WIF were positively associated with BF%, W/H and LDL-C, while energy-adjusted WSF was inversely associated with serum fasting glucose.

Furthermore, the main food sources contributing to total DF intake, including cereals, potatoes, legumes, vegetables and fruits, were one of the concerns relating to the development of OW and OB, and the health consequences investigated in the Belgian population and the Chinese population. Some food sources- derived DF intakes did not have the expected results like total DF intake such as the positive association of WC with fruit-derived fiber in the Belgian population (Table 7.4.). The results from the China study indicated that the associations varied according to the age groups. In the Chinese adult group, BMI and WC were inversely associated with cereal-, vegetable- and fruit- derived fiber intakes, but positively associated with legume-derived fiber. On the contrary, cereal- and legume-derived fiber intakes were inversely associated with BMI and WC, respectively, but fruit- derived fiber was positively associated with WC among school-aged children. Only cereal-derived fiber was found to be positively associated with both Chinese preschoolers' BMI z-score and WC z-score.

Table 7.4. An overview of significant associations of energy-adjusted dietary fiber (g/d) among European adolescents, and fiber from main food groups (g/d) among Belgian population and Chinese population with obesity and presented in the thesis

Food items	**European adolescents †**							
	BMI z-score		**Body fat (%)**		**WHR**		**W/H**	
	B	***P***	**B**	***P***	**B**	***P***	**B**	***P***
Energy-adjusted total dietary fiber	NS		NS		NS		NS	
Energy-adjusted water-soluble dietary fiber	NS		1.7	0.005	NS		0.009	0.014
Energy-adjusted water-insoluble dietary fiber	NS		0.706	0.014	NS		NS	
Food groups	Belgian population				Chinese adults‡			
	BMI		Waist circumference		BMI		Waist circumference	
	B	*P*	B	*P*	B	*P*	B	*P*
Total fiber	NS		-0.118	<0.001	NA	NA	NA	NA
Cereals	NS		NS		-0.147	0.005	-0.146	0.005
Potatoes	NS		NS		NS		NS	
Legumes	NS		NS		0.066	0.009	0.067	0.008
Vegetables	NS		NS		-0.040	0.021	-0.041	0.018
Fruits	NS		0.731	0.001	NS		-0.388	0.049

NA: not available, NS: not significant

†Energy-adjusted water-soluble dietary fiber was inversely associated with serum glucose (B =-0.010 , P=0.020), but weakly positively associated with serum LDL-C(B =0.031 , P=0.047).

‡ Cereal-derived fiber was positively associated with BMI z-score (B =0.403, P=0.018) and waist circumference (B =0.113, P=0.010) of Chinese preschoolers, but inversely associated with BMI of school-aged children (B =-0.327 , *P*=0.011). Waist circumference of school-aged children was inversely associated with legumes-derived fiber (B =-0.093, P=0.023), but positively associated with fruit-derived fiber (B =1.6, P=0.015).

1.3. The associations between dietary protein, fiber intakes and socio-economic status and lifestyle factors in Flemish preschoolers

As explained before, SES and lifestyle factors were investigated instead of indicators of OW and OB in the Flemish preschoolers. Hence, the findings based on the results in the *Chapter 3.3.1. and 3.3.2.* show that parental SES was associated with preschooler's protein and fiber intakes, maternal education and employment in particular. For example, DF intakes from high-nutritious foods (e.g. cereals) were consumed more in preschoolers with higher educated mothers and less DF intakes from energy-dense, low-nutritious foods in preschoolers with one or both parents being employed. In addition, preschoolers with (lower) secondary educated fathers had lower protein intakes from quality foods such as animal sources, dairy, meat, vegetable, and fruit, whereas preschoolers with (lower) secondary educated mothers consumed less plant proteins, bread and cereal-, and vegetable-derived proteins and more poultry- and fish-derived proteins, compared to those with higher educated fathers and mothers.

In the *Chapter 3.3.1*, preschoolers and parental lifestyle factors were examined on animal, plant, and food group-specific protein intakes. Compared to preschoolers with high physical activity levels, preschoolers with low and moderate physical activity had lower animal and plant protein intakes. Furthermore, significantly higher potatoes and grains-, and fish- derived proteins were reported for preschoolers of smoking mothers and fathers, respectively, compared to those of non-smoking mothers and fathers.

2. DISCUSSION OF FINDINGS

2.1. Dietary intakes

2.1.1. Total energy and total protein

The worldwide increase in the prevalence of OW and OB is of great public health concern [141;165;469]. Dietary intake has been considered as a factor associated with chronic diseases such as CVD and T2D [222;293;430]. Epidemiological studies indicate that diets containing high amounts of fruits and vegetables are associated with lower risk of CVD [222].

In this thesis, dietary intake is not only compared between Chinese and European/ Belgian populations, but also among Eastern and Western dietary intakes and dietary behaviors. Compared to the European populations presented in this thesis, total energy intake was lower in Chinese preschoolers and school-aged children, except for female Belgian adolescents. Total energy intake in Chinese male adults was similar as it in the Belgian male adults, but higher in female Belgian adults. The age group factor might be the reason why total energy was higher in female Chinese adults.

Total protein intake of the Belgian population (15.4%), Flemish preschoolers (15.4%) and European adolescents (15.7%) contributed more to the total energy intakes than in the Chinese population (12.3%). Total protein intake contributing to energy intake in the Chinese population, taking into account the age factor, was considerably lower than the peer groups of the target European populations. Likewise, total protein intake was found to be extremely lower in all the age groups of the Chinese population than in the Belgian population, Flemish preschoolers and European adolescents, except for the inhabitants living in the SM region. However, European adolescents participating in the HENELA-CSS were from one city of the country, therefore, the dietary intake and dietary behaviors of European adolescents is not representative for the entire European adolescents. The difference in dietary intakes between the Chinese and the European population is described based on the literature [31;428]. Total protein intake was much lower in the Chinese population than in the peer groups of European populations such as their Spanish peer (10-75 years) [432], Western European adolescents [412], Southern European adolescents [113], Spanish children and adolescents (2-17 years) [431] and European children (2-9 years) [67]. One interesting observation is that Chinese preschoolers had a relatively similar total protein intake as the British and German preschoolers [32;194]. It might be explained by China's One Child Policy. China's One Child Policy, officially started in the 1980s, has developed unique family culture for raising one child with highly intensive care and attention [564]. "Little emperor" or "little sun" are the special names for those children from one-child-families who increasingly become the center of attention in the household, then, as a result, parents and grandparents tend to spoil them by feeding more and better foods with a large consumption of high energy-dense foods, boys in particular [564]. A significantly higher protein intake, including animal and plant protein, was only observed in school-aged boys in this thesis. However, after adjustment for energy intake, statistical significance disappeared due to too much dietary intake among boys. It is noteworthy that the small sample size of Chinese preschoolers may lead to the insignificant results.

Concerning the food sources contributing to total protein intake, animal protein intake was the main contributor (total population, around 67%) in the European populations in this thesis This finding is completely the opposite of the results from CHNS (plant protein contributing around 74% to total protein, urban: 65%, rural:78%). Evidence shows that the European diet is constituted of high amounts of animal sources with low vegetables and fruits [113;284;387;412;432]. Although nutrition and lifestyle transitions have been influenced by the negative Western style, Chinese still kept more healthy dietary habits than the European populations. However, the nutrition transition shifting from the traditional classic Chinese pattern including high intakes from cereals, vegetables and fruits with few animal foods towards a Western dietary pattern with high intakes of energy, fat, animal sources and sugar was observed in the dietary habit region of SM, which is one of the richest regions in China (*Chapter 6*). Not only food habits, culture and lifestyle, but also the economic factor, which influenced the transitions indirectly, led to the above findings. Hence, the nutrition transition appears gradually more in the urban areas and big cities. The inhabitants living in the SM region consumed significantly higher total and animal proteins, preschoolers and school-aged children in particular (plant protein contributing to total protein, preschoolers: 56%, school-aged children: 59%, adults: 67%), compared to other dietary habit regions. Interestingly, total protein intake of Chinese preschoolers in the SM region was close to the intake of Flemish preschoolers, but total protein intake of Chinese school-aged children and adults in that region exceeded the intake of peer groups in the Belgian population (*Appendix 12*). Whereas, the huge difference of animal and plant protein intakes between the Chinese population and the European populations still existed.

2.1.2. Total fiber

As discussed before, Chinese had more healthy dietary intake through the whole country, compared to Belgian/European populations. However, DF intake in the whole target Chinese population was below the Chinese dietary intake guidelines and international recommendations, similar as the European populations in this thesis.

DF intake among the Chinese population was similar as the intake of peer groups among the Belgian population. However DF intake in Chinese preschoolers and school-aged children was much lower than it in Flemish preschoolers and European adolescents. In addition, DF intake was observed to be relatively higher in the FM region in China, due to a high consumption of flour processed products. Crude DF intake in the SM and FM regions was observed higher than the Belgian population, but still lower than Flemish preschoolers and European adolescents. After adjustment for energy intake, DF intake still maintained lower in Chinese preschoolers than Flemish preschoolers, but also lower in Chinese adults than Belgian adults as well. However, energy-adjusted DF intake in Chinese school-aged children was similar as the intake in European adolescents, but considerately higher than Belgian adolescents (15-18 years). Too much dietary intake resulted in a higher crude DF intake among Belgian and European adolescents, but lower energy-adjusted DF intake. Preschoolers get more attention from their parents and school teachers [268], which guarantees the quality of consumed foods and less consumption out of home/ school. Whereas, adolescents have more accesses to get their preferred foods [360]. Compared to the Belgian adults, lower energy-adjusted DF intake was found in Chinese adults. The potential reason might be over-preparation and small portion size which result in a lower daily DF consumption. Moreover, the diverse food consumption databases were used in the different target populations, so the various definitions of DF can result in the discrepancy of total DF intake. For example, the estimated total DF intake was calculated based on the estimated WIF intake in CHNS. The different dietary cultures and dietary habits are the potential factors as well indicating that popular foods and portion size may result in different amount of intakes.

2.1.3. Main food sources contributing to protein and fiber intakes

Evaluation of the main food contributors from food sources reflects the difference in the Chinese and Western dietary patterns between the Chinese and the European populations. Contributions from specific food sources based on the results reveal the significant difference of dietary structures and dietary patterns, dietary cultures and dietary behaviors presented in *Chapter 3, Chapter 4, Chapter 6, Appendix 4, Appendix 5, Appendix 6, Appendix 8, Appendix 9, Appendix 14 and Appendix 15.* Although the nutrition and lifestyle transition were observed in China, the differences remained across China and Belgium/Europe. Compared to the European populations, Chinese people consumed more rice, legumes, vegetables and eggs with less consumption of meat and meat products, and dairy products, which was completely opposite to the European dietary culture [195]. Due to the majority of Chinese participants living in rural areas, it reflected the traditional Chinese dietary pattern. While European populations including Flemish preschoolers, the Belgian population, European adolescents, had a high consumption of meat and meat products (red meat and processed meat in particular), bread and breakfast cereals, dairy products, potatoes and energy-dense, low-nutritious foods. Although the results of HELENA-CSS cannot be representative for the dietary habits of the whole European adolescent population, evidence reveals that meat and meat products were the largest contributors to total protein intakes in European adolescents, and less from vegetable and fruit sources [113;303;432]. Hence, that food composition reflected the typical Western dietary pattern.

Chinese dietary culture and dietary habits resulted in different amount of intakes. A typical Chinese meal usually consists of rice and two/three/four side dishes, which are made of fresh seasonal vegetables, fresh seafood or bite-size portions of meat (red meat and poultry). The different meat preparation usually mixes with vegetables, nuts and small pieces of meat and meat products. So the small portion of meat intake as well as the different way of meat preparation can explain the extremely lower intake of animal proteins in the Chinese population (Chinese: 3-6: 16.3%; 7-17: 17.5%; ≥18: 16.4%; Belgian population: 35.0%; Flemish preschoolers: 34.7%; European adolescents: 40.1%) and a higher intake of plant protein. Results from the Belgian population and Flemish preschoolers show that red meat was the largest contributor (Belgian population:18.2%; Flemish preschoolers: 15.6%), which are consistent with previous nutrition studies in Europe [291;415;432]. High red meat consumption is one of the key features of the Western dietary pattern. Although no information was available in the Chinese population in this thesis, recent reports from longitudinal studies show that white meat from poultry intake is the key feature of the meat consumption in the traditional Chinese dietary pattern as it was a main contributor to the meat consumption [467;569]. The different patterns of meat consumption are related to high and low prevalence of OB in Europe and China/Asia, respectively. Although fish-derived protein was not a main contributor to animal protein in Flemish preschoolers and European adolescents, data reveals no huge difference of fish contribution to protein between the Chinese population (Chinese: 3-6: 3.5%; 7-17: 4.2%; ≥18: 4.6%) and the Belgian/European population (Belgian population: 5.9%; Flemish preschoolers: 3.0%; European adolescents: 4.6%), because of less frequency of the fish consumption than red meat and poultry.

As discussed above, the difference of dietary habits and food preparation supports our finding that vegetables were the largest contributor to the total DF intake (Chinese: 3-6: 37.8%; 7-17: 41.8%; ≥18: 43.3%) and the top 4^{th} contributor to total protein (Chinese: 3-6: 6.9%; 7-17: 7.6%; ≥18: 8.1%) in the Chinese population. Whereas vegetable intake was observed contributing much lower to total protein and fiber in the Flemish preschoolers (protein: 2.4%; fiber: 11.8%), the Belgian population (protein: 2.9%; fiber: 14.4%) and European adolescents (protein: 3.3%; fiber: 10.0%), which is in line with Western dietary behaviors [291;415;432;523;579]. Besides dietary cultures and dietary behaviors, the availability of diverse vegetables is an important factor as well.

Cereals and cereal products, on one hand, were the top contributor to total protein intake in the Chinese population (3-6: 48.1%, 7-17: 50.6%, ≥18: 51.4%) and one of the main contributors to total protein intake in the European populations (Belgian population: 19.3%; Flemish preschoolers: 14.2% including grain, pasta and flour; European adolescents: 17.7% including grain, pasta and flour) included in this thesis. On the other hand, cereals and cereal products were one of main contributors to DF in all the target populations (Chinese, 3-6: 38.5%, 7-17: 37.7%, ≥18: 37.9%; Belgian population: 33.8%; Flemish preschoolers: 31.4% including grain, pasta and flour; European adolescents: 35.3% including grain, pasta and flour). Although cereals and cereal products were one of main contributors in all the target populations, the contributing sources of the Chinese population were different from Belgian/European populations. According to dietary culture and dietary habits, rice, noodle and steamed bread as the main foods in the Chinese diets are equivalent to bread, potato and pasta in the Western diets [466], which can explain that potato was one of the main contributors to total protein and total fiber in European populations in this thesis. Noodles and flour- processed products are consumed more in Northern China and larger amounts of rice are consumed in Southern China. Whereas, bread and breakfast cereals, the favorite and important foods for the European people, are consumed frequently. So it is obvious to explain why cereals and cereal products contributed most to plant protein and DF in the Belgian population, Flemish preschoolers and European adolescents. Compared to the percentage of contribution from food sources, significant discrepancy was observed in all the peer groups between the Chinese population and Belgian/European populations. In addition, the classification of cereals and cereal products into food groups was different in the target populations. For example, the group of cereals and cereal products of the Chinese population and the Belgian population included all the cereal products such as breakfast cereals, flour products and grains. However, in the studies of Flemish preschoolers and European adolescents, grains such as pasta, noodle and rice were classified into the group of potatoes and grains. That can explain the slightly different ranking among European populations. Considering grains and flour, cereal-derived protein was the 2nd largest contributor to total protein in European adolescents.

One of the important findings is that dairy- derived protein contributed significantly less to total protein intake in the Chinese population, adults in particular (Chinese: 3-6: 2.8%; 7-17: 1.2%; ≥18: 0.7%), compared to the European populations (Belgian population: 17.7%; Flemish preschoolers: 32.0% including cheese; European adolescents: 17.2%) in this thesis. The nutrition transition has been proven to occur in China, but dairy products are not considered as an important dietary food item in the traditional Chinese dietary pattern yet [467;569]. Although consumption of milk has increased gradually due to the nutrition transition [360], the huge gap between the amount of consumption and the recommended amount, proposed by the Chinese Dietary Guidelines (2007) still exists [144]. One reason is that the majority of participants living in the rural areas preferred traditional soya-milk and soya-products instead of milk and milk products due to long-term traditional dietary habits and acceptance of new Western food items. In addition, Chinese do not consume large amounts of dairy products such as milk and cheese because of lactose intolerance, adults in particular [404]. Conversely, dairy products such as milk and cheese are one of the most frequent daily drinks and foods in the Western world. For example, milk and flavoured milk drinks contributed the top 2nd and the top 3rd to total protein intake among Flemish preschoolers, respectively. While flavoured milk drinks were the top 6th contributor to total fiber intake among Flemish preschoolers, due to high frequent consumption. Similarly, the subgroup of milk and buttermilk, and the subgroup of cheese contributed the top 3rd and 4th to total protein intake among European adolescents.

The significantly different findings in legume consumption between the Chinese population and the Belgian/European populations are consistent with recent studies [291;415;432;579], as legumes, soya products in particular, are an important type of food for the Chinese dietary pattern, but not for the Western food pattern [467;569]. Legumes were reported to contribute much more to total protein (Chinese: 3-6: 7.7%; 7-17: 8.2%; ≥18: 8.7%) and fiber (Chinese: 3-6: 7.0%; 7-17: 6.6%; ≥18: 6.8%) in the Chinese population than the other target populations (Belgian population, protein including soya: 0.44%, fiber including soya: 0.87%; Flemish preschooler, protein: 1.1%, fiber:2.0%; European adolescents, protein: 1.4%, fiber: 3.8%). A previous comprehensive study among the European population reported that legume consumption is a minor contributor to the daily dietary intake among the entire European populations [500]. A longitudinal study conducted on the Spanish population shows that the trend in intakes of legume protein and legume fiber kept low for the whole study period and no significance was observed after 10 years [432]. Apparently, soya and soya products such as tofu and soya-milk are one of the most popular and favorite foods among the Chinese population providing similar nutrients as dairy products, which is a new introduce from China to the Western world. The level of popularity can be an important factor resulting in huge different amount intake between the Chinese and European populations.

2.2. Associations of animal and plant protein, and fiber intakes with indicators of overweight, obesity and chronic diseases

Over the past decades, along with rapid economic growth and social changes, the Chinese people have experienced remarkable shifts in its traditional Chinese dietary pattern and disease patterns such as OB and chronic diseases [556;560]. This dietary pattern change has been leading to an increased consumption of animal food sources from pork, poultry and eggs and a decreased consumption of vegetables, fruits and legumes [573;574]. Although the remarkable shift, Chinese still kept the traditional Chinese dietary pattern. Therefore, the second objective of this thesis is to study more in details of the association of protein and fiber intakes and intakes from food sources with indicators of OW, OB and OB-related chronic diseases in the target populations. The purpose is to evaluate the associations of these two dietary patterns in their local populations, Chinese population in particular due to the shirting changes.

2.2.1. BMI and body composition

2.2.1.1. Total protein and total fiber intake

Generally, the intake of plant protein was associated with lower BMI and building body composition, while animal protein intake was found to be positively associated with BMI, WC and BF% among Belgian/ European populations [113;209;387;412] and Chinese school-aged children and adults [467;569]. Evidence supports the above findings that, on one hand, plant-based foods [301;365;562] may play a role in a reduction of body weight, building body composition in adults because of less fat intake, on the other hand, foods from animal sources [54;163;561] might increase the risk of OB due to higher intakes of total fat, cholesterol, saturated fatty acids, and higher polyunsaturated over saturated fatty acid ratios. The "Western dietary pattern", rich in red/processed meats, energy-dense foods and sweets, low in vegetables and fruits, is positively correlated with risks of OB, CVD and related cancers [176;205]. The traditional "Chinese dietary pattern"/ "Asian dietary pattern" is known high in vegetables, fruits and grains, low in meat and energy-dense foods. This traditional Chinese dietary pattern is beneficial for prevention of OB, CVD, T2D and related cancers [23;82;205] due to lower fat and cholesterol intakes, but high DF intake. A diet rich in DF has been proved to be associated with lower body weight, lower weight gain and a smaller WC [140;308], because of lower energy and fat intakes and lower glycemic index.

In this thesis, total DF intake was significantly associated with WC in the Belgian population, but not associated with European adolescents and the Chinese population. But energy-adjusted WSF and energy-adjusted WIF were positively associated with BF%/ W/H among European adolescents. The reason is not clear yet. The factors of socio-demographic and lifestyle behaviors need to be taken into account. For example, data analysis of the Belgian population included adolescents, adults and the elderly. European adolescents from eight European cities, which were not representative for the whole European adolescents, might influence the associations, because a diversity of dietary intake still exists within Europe [113;284;387;412]. Likewise, there is also diversities of dietary culture and dietary habits within China. OB and central OB were quite prevalent in the FM, HM, and SM regions in CHNA.

2.2.1.2. Food group-specific protein and fiber intake

The key feature of a "Western dietary pattern", rich in red meat, is positively correlated with risks of OB, CVD and related cancers [176;205]. High consumption of meat, red meat in particular, resulted in positive association of animal protein and animal protein (E%) with BMI and body composition among the Belgian population and European adolescents. Whereas, no association was found among the entire Chinese population, except for school-aged children. Eating small portions of meat together ``Evidence shows that the significant difference between red meat and white meat is the fat content [330]. Compared to white meat, red meat contains more total fat, cholesterol, and saturated fatty acids, and therefore is associated with an elevated risk of OB, central OB and its related chronic diseases [330].

Interesting findings in the Chinese adults reveal that dairy protein intake was inversely associated with both Chinese adults' BMI and WC, which is in line with recent observational studies [39;517]. The possible explanation is the increase of satiety and reduction of appetite. It is obvious that dairy products such as milk and cheese were consumed extremely lower in the entire Chinese population than European populations. As discussed before, lactose intolerance may be a factor for the Chinese population consuming less dairy products. The impact of lactose intolerance on OB is not clear. Based on the findings, low consumptions of dairy can be associated with lower risk of OW and OB, and extremely high intake of milk and dairy products may be positively associated with the risk of OB and its co-morbidities. That might explain the weakly positive association among school-aged children due to excess energy intake from milk, which is consistent with the conclusion of a longitudinal study of adolescents [56]. Calcium is a major mineral component in dairy products. The hypothesis between calcium and body weight has been proposed [571]. Moderate calcium intake from dairy products may decrease intracellular Ca^{2+} regulating adipocyte lipid metabolism and TG storage by increasing lipolysis and suppression of lipogenic gene expression and lipogenesis, which result in diminishing adiposity [571]. Although no information is available among Belgian/European populations in this thesis, one previous cross-sectional study conducted on British women aged 60-79 suggested that women who drank milk had a bigger BMI than those who never drank [289], which is supported by longitudinal studies [136;364]. Marques-

Vidal *et al*. reported that a significantly inverse relationship between milk consumption BMI among healthy Portuguese adults in both genders [322]. The fat content of dairy products, which needs to be adjusted, can be a key factor. Therefore, dairy-derived protein from 'lower'-fat dairy products such as milk and yoghurt may be associated with lower adiposity, with cheese having the opposite association.

On the contrary, fish-derived protein was positively associated with adults' BMI and WC, and school-aged children's BMI in CHNA. One recent cross-sectional study conducted on 1152 adults supported supports the above finding [10]. However, the consumption of fish proteins is believed to benefit on body composition, blood lipids, glucose tolerance and insulin sensitivity [378;509]. One recent meta-analysis indicates that high frequent intake of fish is associated with a lower risk of coronary death [203]. Similarly, fish replacing red meat was reported to be inversely associated with a decreased risk of CHD [224]. 34 OW adults randomized to supplementation with fish protein or placebo tablets (controls) suggested that fish protein supplementation significantly improved postprandial glucose tolerance after 8 weeks, significantly decreased serum LDL-C, BF% and increased percentage of body muscle after 4 weeks, concomitant with increased body weight [509]. Positive association found in CHNA can be explained that fish protein might build body lean mass among healthy Chinese participants. Compared to red meat-derived protein, fish-derived protein is healthy protein providing polyunsaturated fat and less cholesterol. Increasing fish consumption was suggested to be associated with a lower risk of OB and related diseases among healthy Chinese adolescents and adults via less weight gain and modulating TG levels [470;569]. Prospective evidence is limited from observational studies among European populations on OB. A population-based prospective cohort (EPIC-Norfolk) reported that white fish intakes are suggested to be beneficial for reducing risk of diabetes and recommend to consume fish regularly [390].

Due to the different contribution from plant food sources, on one hand, cereals and cereal products were the main contributor to total protein in the Belgian population and European adolescents, while vegetables, cereals and cereal products, and legumes were the main contributors in the Chinese population. On the other hand, cereals and cereal products, and potatoes are the top 2 contributors to total fiber intake in the Belgian population and European adolescents, and cereals and cereal products, vegetables were the top 2 contributors in the Chinese population. Going into depth on food sources, the largest contribution of cereals and cereal products may result in the inverse associations of total protein and fiber intakes in the European populations.

Results found in the food groups show that Chinese adults' BMI/WC were inversely associated with vegetable- and fruit-derived protein/fiber. Based on the contribution to total protein and total fiber, the highest contribution of vegetables can explain the inverse associations in the Chinese adults. Two recent cross-sectional studies suggested that the traditional "Chinese dietary pattern" with a higher consumption of vegetables and fruits resulted in lower BMI and WC among Chinese adults compared to the "Western dietary pattern" with higher consumption of animal sources and lower consumption of vegetables and fruits [467;569]. The 2007 Dietary Guidelines for Chinese recommend consuming plenty of fruits and vegetables to promote nutritional status and prevent from chronic diseases [182]. In addition, WHO recommends to consume at least 400 g of fruits and vegetables per day, excluding potatoes and other tuberous root vegetables, providing a diversified and nutritious diet and prevention of OB and chronic diseases in all the populations [551]. Dietary protein and fiber from vegetables and fruits can increase satiety, decrease subsequent energy intake and decrease energy density. Whereas, fruit-derived fiber was positively associated with WC in the Belgian population and Chinese school-aged children. That can be related to food choice and food decision as some of fruits contain high amount of sugars, besides fiber. Because of the study design, the conclusion of causality cannot be drawn. A long-term prospective observational study reported that a higher level of vegetable and fruit intakes is associated with weight loss and preventing long-term weight gain [344] as that leads to less energy intake and includes intakes of less energy-dense foods. In addition, vegetables and fruits are rich in DF. A population-based prospective study reported an inverse association of vegetables with the risk of T2D among Chinese women, but not fruits [510]. The conclusions are inconsistent with other prospective observational studies [196;506], because no energy intake increased cannot impact on weight loss and body composition. Moreover, some factors need to be taken into account such as age, gender, puberty, physical activity and urban/rural habitual living. The majority of the participants in CHNA were from the rural areas. Physical activity of Chinese participants from the rural areas is heavier than those living in the urban areas. Therefore, dietary behaviors and other lifestyle factors in the rural areas are associated with relatively lower intakes from animal sources, heavier physical activity. Those patterns of dietary behaviors and lifestyles led to a lower prevalence of OB due to a lower energy intake and higher energy expenditure. Although no firm conclusion can be drawn from this thesis, convincing evidence shows that adequate consumption of fruits and vegetables reduce the risk of OB [554].

As discussed above, the group of cereals and cereal products is an important contributor to the intakes of plant protein and DF in all the target populations in this thesis, besides vegetables in Chinese school-aged children and adults. Cereal-derived protein/fiber was found inversely associated with BMI/WC of school-aged children and adults. But no association of cereal-derived fiber with BMI and WC was found in the Belgian population, although cereals were the largest contributor to total fiber intake. Evidence shows that cereals and grains, but not refined grain, are associated with risks of OB, CVD and T2D via decreasing body weight, building body composition and regulating blood lipids and blood glucose concentration in children and adults [7;41;72;333;568] as cereals and grains are excellent sources of protein and fiber into peoples' diets. Refined cereal products contain low nutrition values such as protein and fiber because of food processing, and therefore have high energy density and glycemic index [308]. Look into the contribution from food sub-groups, bread contributed most in the Belgian population and European adolescents. While grains from rice, steamed bread, noodles contributed most in the entire Chinese population. Ready-to-eat cereals are very popular in the Western countries. Greek adolescents consuming ready-to-eat cereals for breakfast were observed to have a lower risk of OW than those consuming other foods [273], which is consistent with the findings from one longitudinal EPIC study on female adults [429]. A higher level of total cereal consumption (pasta, breakfast cereals, rice) affected large weight loss of 2 kg or more, but bread was not associated with weight loss [429]. As known, rice and flour- processed foods are important main foods in Southern and Northern China, respectively. The findings from CHNA shows that mean BMI and WC were lower in Chinese from Southern China (VM), but higher in Chinese from Northern China (FM). Research on rice has not got enough scientific attention. Rice like other whole grain products needs less food processes. Consist with the finding in the Chinese population, recent studies reported that rice as the major staple food is associated with a lower risk of general and abdominal OB in Chinese adults, but dietary protein and fiber from wheat flour (pasta) were positively associated with general OB and abdominal OB [568;569]. In addition, protein and fiber from refined flour-processed foods possible result in positive association with body weight and WC due to energy density [196]. For example, instant noodles were reported to be positively associated with the prevalence of metabolic syndrome because of high energy density, high glycemic loads with refined carbohydrates and high saturated fat content with low nutrition values [438]. The food preparation is one of key factors leading to the difference in energy density, which is related to OB. In China, rice and steamed bread are prepared by steaming and boiling, which keep the foods moist. This clearly explain the relatively lower energy intake compared to the preparation of baked bread. That can explain no association of cereal-derived fiber in the Belgian population, and positive association of energy-adjusted WSF and WIF in European adolescents due to refined flour and energy-density foods.

No association of potato-derived fiber was found with BMI and WC among the Chinese population, although potato-derived protein was observed to be inversely associated with BMI. Potato is not an important food item in the traditional Chinese dietary pattern and contribute minor to total protein and fiber intakes compared to European populations. Small portion size of potato consumption may assist regulating body weight and body composition via increasing satiety and decreasing relatively energy intake. Whereas, potato is an important and common food item to the European populations, and constitutes a typical high-glycemic-index Western diet (e.g., boiled white potatoes:82, baked potatoes:85), therefore, leading to a high-glycemic-load diet. That may lead to positive associations of energy-adjusted WSF and WIF with adolescent's body composition. High-glycemic-index foods affect lower satiety, result in postprandial rises in glucose and insulin concentration, increase carbohydrate oxidation acutely and decrease fatty acid oxidation, thereby being conducive to weight gain and body fat gain [73]. No association of potato-derived fiber was found among the Belgian population. It can be explained that the whole Belgian population including the elderly, who decreases appetite and consumption with aging, was in the final analysis.

As known, legumes, soya products in particular, are very popular and favorite foods the traditional Chinese dietary pattern [467;569]. Legumes contain high nutritional values, high in fiber and a good source of protein with low energy density, which reduces the glycemic response and affect satiety [407]. Unexpectedly, legume-derived protein and fiber were related to higher BMI and WC in Chinese adults, although legume was the largest 3rd contributor to total protein and total fiber in the Chinese population. Most studies on the associations of legumes and OB are clinical trials, which refute the findings in the Chinese studies [11;14;125;301]. The contribution from legume-derived protein and legume-derived fiber in the European populations in this thesis was extremely low. Therefore, the contribution to association was minor in the Belgian population and European adolescents. Recent cross-sectional studies reported that the traditional Chinese foods with a high consumption of soya-beans were inversely associated with BMI, WC and risks of CVD [467;569]. Maskarinec *et al*. showed that having beans in the diet, including legumes, tofu and soya products, was inversely related to women's BMI after adjustment for daily energy intake [323]. The possible reason is that a soya or a soya-protein-enriched diet may be better at loss of weight and reducing body fat, but building muscle mass by providing an increased abundance of amino acids will increase muscle protein synthesis in obese people [76;125]. Another potential factor can be explained by a high frequent consumption offering more energy intake from the sources of legumes and tofu, and soya products. A high amount of essential and branched-chain amino acids from plant proteins has been reported to be responsible for the amino-acid stimulation of muscle protein anabolism and promote muscle protein synthesis [462]. That can explain the positive association in Chinese adults because of building muscle mass. In addition, legume-derived fiber decreases energy availability and the energy density. Compared to meals, OW/OB subjects consuming legumes (4 servings per week) within a low-energy diet (30% energy restriction) for 8 weeks had a greater weight loss than those having the energy restricted control diet without legume consumption [210]. Anderson *et al*. evaluated the effects of soya-based and casein-based meal replacements on weight loss and body composition in obese women [18]. Both soy and casein consumers lost body weight and body fat from their initial baseline data, with no significant difference between two treatments after 16 weeks. Some recent studies clarify that total, animal and plant proteins are inversely associated with only OW and obese individuals, but not normal healthy individuals, which could be a deeper insight into prevention of OB [1;36;125;159;301;562]. The potential reason is that OW/obese people need dietary protein to increase free fat mass and decrease fat mass by decreasing energy intake [530]. On the contrary, normal healthy individuals or lean individuals need dietary protein to prevent muscle loss instead of losing body weight [207]. Although the results are inconsistent, WHO (worldwide), WHO (European Region), and the Chinese dietary guidelines all strongly recommend to increase legume consumption for improving nutritional and health status, which can prevent OB and chronic diseases [182;551;552]. The U.S. Food and Drug Administration suggests to consume 25 g of soya protein per day as it may reduce blood cholesterol and the risk of heart disease [166].

2.2.1.3. Dietary protein and fiber intakes related to children's obesity

Some positive associations were found in Chinese preschoolers and school-aged children between meat- and fish- derived protein, and cereal-derived fiber intakes and BMI, cereal- and fruit- derived fiber, and dairy-derived protein intakes and WC. Childhood OB in China has been rising rapidly over the past 20 years. The highest prevalence of childhood OB was reported in Northern China and well-developed regions, which is related to dietary intake rich in energy and animal sources [172]. The findings of CHNA in this thesis shows that the higher prevalence of OW and OB, and abdominal OB in preschoolers and school-aged children was observed in Northern China (HM and FM), but not in the relatively rich region (SM: Jiangsu province). The dietary intake in HM is comparatively heavy diets full of fat and animal sources because of geography and climate. While the dietary intake in FM is rich in flour-processed foods. Flour-processed foods, discussed above, are related refined flour foods. In addition, China's One Child Policy may be an important factor related to positive associations among Chinese preschoolers and school-aged children because of over-nutrition and over-attention [114]. The potential factor is the pre-adolescence and adolescence, as adolescents are considered as special population in a critical and dynamic period with hormone growth, building muscle, being responsible for behavior and body weight [97]. During this special period, adolescents need more energy and nutrition to develop body composition and build muscle. That may explain the positive association of WSF and WIF found in European adolescents. Gunther *et al.* suggested the significant difference between animal and plant protein impacting on the children's hormone growth [194]. A high animal protein intake during mid-childhood might be associated with an earlier pubertal growth spurt peak height velocity, while a higher plant protein intake, conversely, could delay puberty [193], although data in this thesis could not check this association due to study design.

2.2.2. ***Biomarkers***

Biomarkers were used to investigate the association between protein intakes and OB related indicators in European adolescents in HELENA-CSS. Serum HDL-C was found to be weakly positively associated with animal protein intake, and serum glucose was inversely associated with energy-adjusted WSF. Evidence shows that increasing protein intake results in improvement of serum lipids and insulin response [212;300;411;459]. Whereas, plant food intake was suggested to have more benefits on blood lipids in children and adolescents than animal sources [47;154;155], due to low consumption of total fat, saturated fat and cholesterol. Plant foods are rich in DF, which is responsible for altering glycemic and insulin response [324;471]. However, inconsistent effects of plant-based food intake on OB were reported [258]. More frequent consumption of ready-to-eat cereal was not significantly associated with blood lipid levels in Greek adolescents [258]. However, inverse associations were observed with blood glucose in European adolescents in this thesis. The reason of unexpected positive association in this thesis is not clear. Pre-puberty and puberty might influence the association as puberty leads to different eating behaviors and lifestyle, hormone growth, and development of body weight and body shape. In addition, data of adolescents from all the eight countries in the HELENA-CSS was put in the same model for data analysis. This might affect the association because of the dietary diversity, although the effect of cluster country was controlled in the model. The small sample size of serum biomarkers may be an important influencing factor leading to the weak associations, besides the confounding factors including gender, age, puberty status and regions, which also can explain the unexpected weak associations of biomarkers. However, the strongly statistical power shows that the results are valid.

2.3. Associations of dietary protein and fiber intake with socio-economic status and lifestyle factors in Flemish preschoolers

Investigating the association of protein and fiber intakes with SES and lifestyle factors is the 3rd objective in this thesis. Diet quality is strongly influenced by SES including occupation, education and household income. The findings in this thesis show that SES of parents including education and employment is importantly associated with high quality dietary intake of their children. Evidence shows that children from parents with a higher SES are more likely to have more 'healthy' dietary patterns (more cereals, fruits and vegetables) in the Western countries, whereas more 'unhealthy' dietary patterns (energy-dense, low-nutritious foods) are found in families with a lower SES [138;139;536], which supports the findings in this thesis. For example, Flemish preschoolers with higher educated mothers consumed more cereal products, potatoes, grains and vegetables than those with less educated mothers. Compared to maternal education, paternal education is more associated with consumption of animal food sources. Flemish preschoolers with higher educated fathers consumed more animal protein, dairy- and meat protein, compared to preschoolers with less educated fathers. Preschoolers consumed more fruit and fish with increasing a maternal educational level, while the snack consumption became more frequent with decreasing a maternal education level [358]. Therefore, mother is more responsible for nutrition and healthy dietary intake for children [358]. While, fathers are probably more responsible for the household income. The reason is not clear why the paternal education level is associated with intake from animal sources in preschoolers. One recent study reported that paternal control was associated with lower percentage of kilocalories from carbohydrate, but greater percentage of kilocalories from animal sources [266].

Dietary behaviors are influenced by lack of knowledge as well. Knowledge gained in the classroom was reported not to satisfy to change behaviors [394]. As discussed above, the parental level of education and knowledge, maternal in particular, plays an important role in the development of children's eating behavior and lifestyle [49]. Well-educated parents have better awareness of healthy nutrition, understand nutritional messages and adopt healthy dietary behavior better than less-educated parents. Thus, parents having better nutritional knowledge, mothers in particular, may translate nutrition knowledge into healthier food choices for the entire family, providing better-quality food items [138]. Well-educated parents have more consciousness of planning family meals, making healthy food patterns, and encouraging the entire family to have healthy dietary habits and lifestyle than those less educated parents. Those require family organization and planning, appropriate monitoring, and effective management of the family's daily routines and activity, and child behavior. Thus, children from well-educated families with optimal parenting behaviors are likely to have awareness of building healthy dietary habits and lifestyle.

The 'unhealthy' Western dietary pattern leads to childhood OB because of higher intake of sugar, fat from fast foods, snacks and confectionery [409]. The association between low SES and childhood OB might be the consumption of low-cost, energy-dense, low-nutritious foods. Price and income can affect food choices, dietary behavior and quality of diet. So families with a high SES purchase more high-quality food such as lean meat, fish, vegetables, fruits and nuts than families with a low SES [138]. Flemish preschoolers living with both-unemployed parents were found to consume more DF from energy-dense, low nutritious foods than those living with both-employed parents and one-employed parent. Whereas, Flemish preschoolers with one-employed parent had significantly higher vegetable-derived protein than those with both-unemployed parents, although no significance was found in preschoolers with both-employed parents. The potential fact may be that children got less attention from both-employed parents and consumed regularly out of home because of lack of time [211]. Children were observed to have less healthy foods out of home, but their home-meals were predominantly healthy [211]. Therefore, parental involvement plays a critical role in establishing and promoting children's health behavior and dietary habits [394], which in turn affect their children's weight status. For example, parental communication, maternal communication in particular, with their children is considered as an important involvement to build children's nutritional behavior [264;440].

Although SES factors were not investigated in the Chinese population, economic status is obviously related to dietary intakes, based on the results of CHNA. High family economic status resulted in higher intakes from animal sources in China [436;574], while, high family economic status in Europe is associated with more healthy dietary patterns [138;536]. Opposite to Western countries, Chinese children, boys in particular, are over-fed, which often happens in wealthy and well-educated families in China because of China's One Child Policy. Parents' over-attention and over-protection can result in over-feeding and over-nutrition, which caused the rapid increasing childhood OB in China. Hence, cultural factors are important determinants impacting on food choice and food quality, besides financial and education factors. Parents' right nutrition knowledge and behaviors can positively translate the healthy nutrition message to children [42;211;357]. Therefore, parent involvement in a children's health promotion program including nutrition education and lifestyle are recommended at home or school bases [394], which can positively establish the view of health and nutrition for the entire family.

In addition, lifestyle-related factors such as children's physical activity were reported to influence children's dietary behavior in this thesis. Flemish preschoolers having light/moderate physical activity consumed significantly less total animal/ plant proteins, compared to those having heavy physical activity. Physical activity and inactivity of children were reported to be significantly associated with dietary patterns in school-aged children [111]. 'Healthy' dietary patterns are more associated with spending more time on physical activity, whereas more 'unhealthy' dietary patterns were associated with a sedentary lifestyle [111]. Children having a sedentary lifestyle are more likely to have unhealthy dietary patterns, consuming more energy-dense, low-nutritious foods, such as bakery products, salted and sugary snacks as well as soft drinks [24]. Children's eating behaviors and lifestyles are evidently affected by parenting behaviors [24;111;339]. Parents are important idols and teachers for their children. So, children are more likely to copy and translate parents' lifestyle into their life. Children living with both parents, who are more physically active, are more likely to be more physically active than those children living with parents having inactive lifestyle [110]. Parents spending a considerable amount of time on physical activity together with their children are recommended to positively encourage children's physical activity behaviors [442]. Mothers probably spend much more time with their children than fathers. Thus, maternal permissive parenting is believed to be associated with higher levels of physical activity in their children [240].

Furthermore, parental smoking status was observed to be associated to Flemish preschoolers' dietary behaviors. Few studies have been investigated the relationship between parental smoking status and children's dietary behaviors. Evidence from available studies shows that parental smoking lifestyle may affect children's dietary behaviors and dietary intakes [112;252;410]. Children of non-smoking parents had a more healthy dietary pattern than those of smoking parents having more energy intake from saturated fat and less DF intake [112;252;410]. Parental smoking status is associated with their SES such as occupation and education levels, mother in particular [112;410]. Smoking parents, especially for heavy smokers, are more often observed to have lower SES [112]. Paternal smoking was positively associated with fish-derived protein intake of Flemish preschoolers, while, maternal smoking was positively associated with protein from potatoes and grains. Flemish preschoolers with their smoking mother might have a higher consumption of French fries and chips compared to preschoolers with non-smoking mother. The reason is not clear about the association of paternal smoking status. As mothers are more important to be responsible for their children's dietary intake and dietary behavior, maternal smoking status can affect children's dietary intake more than paternal smoking status [106]. In addition, the category of maternal/paternal smoking status of Flemish preschoolers was not divided into non-smoking, light smoking and heavy smoking because of relatively small number of smoking mothers and fathers. Moreover, this cross-sectional study can't draw causality. The significant association can indicate that the parental lifestyle are associated with their children's dietary intake and dietary behaviors.

3. METHODOLOGICAL CONSIDERATIONS

3.1. Dietary assessment

A key objective of dietary assessments is to estimate the average habitual and usual long-term food or nutrient intake for a group of individuals [417]. It is important to note that no dietary assessment method is perfect and measurement of habitual food consumption is one of the hardest tasks to collect accurately because of two basic problems. They are memory dependence of food intakes, and the conversion of these food intakes to nutrient and energy data.

In this thesis, two dietary assessment methods including a 24-h dietary recall and a non-consecutive EDR were used to collect dietary intakes in four independent populations. Both assessments have pros and cons. Some limitations still need to be taken into account.

The 24-h dietary recall is considered as a good dietary assessment method with a high response rate. Detailed dietary information can be easily obtained as well. And respondents should be able to recall what they have consumed in the last 24 hours. However, the 24-h dietary recall is not considered to be representative for the habitual diet at an individual level. For example, a 24-h dietary recall cannot collect data of quantifying proportions of non-consumers for particular food items. The 24-h dietary recall is adequate to collect dietary information in a large group and estimate group mean intakes. Multiple 24-h dietary recalls were used in the target large-population based surveys. 24-h dietary recalls with a guided computer-assisted program, were used to collect dietary intakes in the BNFCS, HELENA-CSS and CHNA. A guided computer-assisted program can assist to remind the respondents of consumed foods. In addition, colored photographs for different food portion sizes were provided to the participants during the interviews in the BNFCS and HELENA-CSS, in order to quantify a correct food portion intake. Detailed data such as brand name and cooking method can be described.

An EDR is similar to a 24-hour recall. It consists of a detailed description of food and drink consumed, such as brand name, cooking and preparation methods, over a period (usually three to five days). Multiple EDRs can also only reflect a short-term dietary intake rather than a usual intake, which may not be representative of individual's habitual long-term diet. A high degree of respondent cooperation is necessary. So the act of recording may alter the habitual long-term diet.

Therefore, regarding both dietary assessment methods, assessing more repeated randomly interviews by a well-trained dietician/researcher will obtain more reliable and valid information. However, it is often difficult to implement completely due to several factors such as budget and drop-out.

3.1.1. Between- and within-individual variation

Dietary intakes vary from day to day for every individual through variations in meal patterns. The household composition, foods availability, foods consumed out of home, working patterns, school patterns, home patterns, workday patterns or weekend patterns can influence the meal patterns. Then variance of the mean intake of a nutrient must be dependent for between- and within-individual variation, which can influence the validity of intakes. However, the methodologies such as study design, data collection and statistical analysis can adjust the estimated dietary intakes of between- and within-individual variation. All the studies included in the thesis had collected 2-3 days of dietary intakes in each large-scale based populations. A large scale sample size can guarantee that the dietary intake is representative for the entire population, indicating that the impact of between-individual variation on mean intakes has been maximally reduced. Hence, the valid dietary intake of each population can be estimated accurately.

In addition, the valid each individuals' usual nutrient intakes between-individual variation must be relative to within-individual variation [184]. There is greater variation within-individual than between individuals for most nutrients [46]. As mentioned before, all the dietary intakes in this thesis were based upon 2-3 days intake, and besides data collection, statistical methods such as MSM were used to correct for within-individual variation with exception for the data of the Chinese population. The quality of dietary intake in the Chinese population was checked by comparing an individual's average daily dietary intake, which was described in *Chapter 6.* High quality of dietary intake can decrease variation at individual level. The large study sample size of CHNS is sufficient to reduce the effect of between-individual variation on group mean intakes.

3.1.2. Seasonal variation

Diets can vary widely not only between individuals and within individuals, but also diets may vary due to seasonal effects such as winter and summer. In the cross sectional health and nutrition surveys in this thesis, this factor was taken into account as 24-h dietary recalls were used to collect dietary intake throughout the year to reduce the effect of seasonal variation. Moreover, interviews of 24-h recalls were randomly allocated to different days of the week and weekend, over a 12-month period in an effort to avoid seasonal effects, which can reduce within-person variation in the meantime as well. For the 3-d EDRs, seasonal effects could not be ruled out at the start of the fieldwork because the fieldwork was carried out only during autumn and winter. The research team determined beforehand the days to record. The respondents would not choose days to record. So, all the days of the week were equally covered. Low seasonal variations influencing dietary intakes in the Belgian population were reported because the widespread availability of most foods all year around [124]. The more interviews are carried out, the more representative data of usual intake in the populations can be captured. However, increasing the number of day recalls / records per individual may increase the risk of rate of drop-out. Therefore, 2-3 interviews were considered as acceptable times for collecting the maximum valid data.

3.1.3. Misreporting

All methods used to assess self-reported daily dietary intakes have several limitations in terms of the accuracy of the portion size estimation and description of consumed foods [63]. The potential bias existing from the beginning, such as SES or some physical factors, can result in misreporting of food consumed. In the low SES families, participants tend to over-report their dietary intake rather than the accurate dietary intake. Then finally, the portion sizes are over-estimated. There is also a reported bias for the preschoolers and children, whose dietary intakes were reported by parents/proxies.

It is known that under-reporting of dietary intakes of energy, meat, snacks or other energy-dense, low-nutritious foods is more common in OW/ obese individuals compared with their lean counterparts [206]. Conversely, fruits, vegetables and legumes are more easily found over-reporting among OW and obese subjects. So the estimates of associations of dietary intakes and health outcomes may be biased in either direction. In addition, under-reporting is also related to gender, age, smoking, SES, emotional states, physical activity and psychological status. For example, women are more likely to underreport energy intake than men [267], whereas, the percentage of over-reporters was largely found in men [250]. Therefore, misreporting is not simply a nutritionist/ researcher's problem, but requires a multidisciplinary approach (including psychology, sociology and physiology) to advance the understanding of misreporting in dietary intake studies. So the dietitian/researcher plays a key role to gain valid and reliable information.

The impacts of SES could be partially avoided when the agreement of anonymity and confidentiality was signed and performed completely during interviews or filling out the questionnaire. Additionally, the importance of honestly recorded dietary intakes leading to correct interpretations of data need to be communicated to the respondents, before the interview.

Although misreporting cannot be avoided, the reliability and accuracy of data collection from four different independent studies can be trusted. Well-trained dietitians/researchers supervised and checked during the interviews and the quality of data was also examined via statistical methods.

3.1.4. The impact of investigators/ researchers

In health and nutrition surveys, investigators/ researchers play a critical role in capturing accurate and reliable reports during the interviews. They are important and necessary to obtain accurate dietary information before ending the interviews. So key instructions are provided by the investigators / researchers, along with a guide to the different nutritional software programs, which are used to assist with nutritional analysis. However, sometimes, investigators/ researchers would confuse participants, which may result in reports with less reliability and accuracy. For example, investigators/ researchers may ask questions twice or more for checking the accuracy, which may lead to participants uncomfortably misreporting the dietary information. Therefore, in order to avoid the unexpected misreports and missing reports, a standard training program is necessary. Thus, investigators/ researchers can be guided to ask questions and analyze the participants' reaction.

3.1.5. Memory dependence

As known, the success of 24-h dietary recalls need to depend on individuals' memory, which also causes misreporting during interviews, the ability of the individuals to give accurate estimates of portion sizes consumed [187;255]. Individuals need to report all the foods consumed at home, at work, at school or somewhere else.

24-h dietary recalls used a picture book with colored photographs describing foods of different portion sizes, which could help participants recall the food consumption in improving the accuracy of food quantification [487].

3-d EDR was only used in the Flemish preschoolers. As discussed before, parents needed to report their child's dietary intake with the help of school teachers. The validity of the dietary intake has been reported elsewhere [227]. The accurate results were not significantly influenced by the biased misreporting.

3.1.6. Parental reports for the children

The dietary intakes of Flemish preschoolers and Chinese children (≤ 12 years) were reported by parents/ proxies, which might result in misreporting of intakes. It's important to note that parents/proxies needed to cooperate with teachers /caregivers at school or elsewhere. It's assumed that parents/proxies got all the information from teachers or caregivers, although it was difficult to observe the children at all the different meal moments. The misestimated portion size may be a possible result. Klesges, *et al.* reported that more reliable and accurate dietary reports were found in younger children because younger children need more attentions from their parents, but still errors were made by parents estimating portion sizes [268], which is in line with Chinese children, in particular for children under the age of 11 years old [572].

In addition, besides the above points of social desirability bias, the role of investigators or well-trained researchers, and memory dependence, parents might unintentionally not report some foods, supplements, snacks or meals eaten outside home because of lack of parental control [42].

The reliability and accuracy of 3-d EDRs for Flemish preschoolers reported elsewhere [227], showed moderately good to good relative validity. However, given the smaller error for parentally reported dietary intake of preschoolers and young children (≤ 12 years) from the

Chinese survey, the data used in CHNS might result in biased exposure of health outcomes, which has been discussed in the *Chapter 6.* As mentioned, the quality of the data collection in China study was checked. Dietary data with significant discrepancies was revisited and inquired about their food consumption to resolve these discrepancies.

3.2. Food categorization

Each national and regional database uses specific food classification systems, based on national / regional criteria, which is related to economic and cultural importance of foods [237]. So national / regional classification is often used. The most difficult to use on an international basis, as the food classes defined may not be applicable to all cultures and populations.

For example, when classifying cheeses, the CIAA Food Categorization Systems [100], an European approved and accepted classification, first differentiates unripened, ripened, processed and analogue cheese; followed by the conditioning, conservation and presence of rind. In Eurocode 2, cheeses are first classed according to their consistency (hard, soft, fresh), then their fat content. However, dairy products such as cheeses (even milk) are not Chinese traditional foods and hardly consumed daily, as they are a new food item introduced from the Western countries. So the classification of cheese for the Chinese population had its own concept and could not follow the European criteria. However, because of China's rapid economic development, the classification of cheese has been adjusted and updated from 1996 to 2011 based on the Chinese Food Categorization Systems. Cheese was classified into six second-subclasses (unripened cheese, ripened cheese, whey cheese, processed cheese, cheese products and whey protein cheese) under the subclass of cheese in the GB2760-2011 version, while the GB2760-1996 version had no second subclasses. Therefore, the classifications are often contradictory, and their existence in the specific population or ethnic groups, shows that no single international classification system can satisfy all the dietary databases/cultures.

The dietary food categorization in this thesis, based on different national /regional guidelines, can be representative for each dietary culture, dietary history, beliefs, dietary habits and characteristics of the populations. However, the different food categorization systems used in this thesis might have influenced minor differences of dietary protein and fiber intakes.

3.3. Food composition databases

Accurate and reliable estimation of energy and nutrient intakes is an important challenge in nutritional epidemiology. Nowadays, most food composition databases are composed using original analytical values, values from the literature and other related databases. Food composition databases, providing an approximation of the energy and nutrient composition of foods, were linked to food consumption data for estimating dietary protein and DF intakes. The data used in the different surveys included in this thesis were all based on national/regional food composition databases (the Belgian food composition table NUBEL (2004), the German food code and nutrient database (version II.3.1, 2011) and the Chinese food composition table (2002). For the Belgian population and Flemish preschoolers, other national food composition tables were used to be linked when some food items were missing in the NUBEL. More details have been described in each chapter.

It is important that linking the correct food composition database can estimate precise and valid dietary intakes at the international level. However, all the food composition databases have their own limitations. Systematic and random errors in the food composition databases including analytical methods, use of incorrect conversion factors, inconsistencies arising from methods of food preparation may arise bias of nutrient estimations and make them incomparable at international level [533]. So at the international level, if nutrients in different countries are estimated by using different food composition databases, the systematic errors can be different and unexpected errors can increase.

All national/regional food composition databases are not updated frequently, which should be maintained manually by humans, because new foods are constantly introduced to the food market and no food composition database can hold complete food items and nutrients (e.g. cheese in China and soya products in European countries). In addition, nutritional interests change with time; for example, most Chinese dietitians and researchers did not require nutrient values for “cheese” before 1996. Whereas, due to economic growth, cheese can be seen in the Chinese urban supermarkets and Chinese living the urban areas have started to consume it. So dietitians and researchers sometimes need values of cheese. Now the values were updated in the Chinese national food composition databases. Therefore, a national/regional food composition database only can have a complete list of foods or nutrients for a short period. Whereas, a complete coverage of all nutrients and food items requires high levels of laboratory instrumentation. When missing nutrient values are from the missing foods, nutrients are frequently taken to be zero resulting in error, or considered as other food groups. However, in fact they are missing values or miss-leading values. Considering the errors caused by missing values, these missing values were avoided to be replaced with “zero” in this thesis. For example, if a food item was missing in the Chinese food composition tables, the missing food item was replaced with a similar food item in order to have high quality of data with no missing values of food items and nutrients. Likewise, the food items consumed in the Belgian population and Flemish preschoolers were first linked to the Belgian food composition table (NUBEL) for the estimation of nutrient intakes. When some food items were not available in the NUBEL or the estimated nutrient intakes were treated as zero, those missing food items were linked to other the tables – in order of importance: the Dutch food composition database NEVO and the USDA food composition database, due to the similarity of dietary intakes. If the food items were still not available in the above food composition tables, the values of similar foods from other food composition tables were used. In the end, if there were still missing values of nutrients, values were used from similar recipes made for ingredients with a full coverage of nutrient data. The food items consumed by European adolescents participating in the HELENA-CSS were lined to the German Food Code and Nutrient Data Base with standardized procedures [257]. For the missed and country specific country specific foods, recipes were created for calculating complex foods, using the BLS foods as ingredients to calculate the total composition of each recipe. Therefore, all the missing values for food items or nutrients were covered by using similar, full covered food nutrient information, foods and recipes.

Moreover, the analytical methods used in the food composition databases are crucial to lead to the different definitions of measurements and analysis, causing various estimations of nutrient intakes. For example, AOAC is widely used to determine and give a value of total DF, whereas, the method of Englyst is used to measure and determine NSP in the UK. Food composition databases in this thesis used the same analytical methods, the Kjeldahl method and AOAC method, to estimate of dietary protein (animal and plant proteins) and fiber (WSF and WIF) intakes, respectively, following the definition proposed by AOAC [28-30]. So, the same chemical analytical method used to determine protein and fiber content in the food composition databases would not impact estimates of absolute intakes.

Although no unique standard food composition database exists satisfying all the populations from different countries, the errors can be decreased and under-/over estimation of nutrient intakes can be adjusted by methods such as data collection, sufficient sample size and statistical analysis,

3.4. Dietary regions in China

Chinese are well known to have a healthy dietary style consuming more vegetables and fruits, and fewer foods from animal sources. Whereas, Chinese have diverse cultural heritages as China is one of the largest country in territorial area and the most populous country in the world. So Chinese have many distinct regional cuisines and dietary behaviors, which are related to availability of resources, climate, geography, history, culture, cooking techniques and lifestyle.

One approach to describing the regional cuisines and dietary behaviors is to note the general characteristics of food preparation and culture by regions: North, South, East, and West. Due to the extremely dichotomous climate, wheat flour prepared foods, such as noodles, jiaozi (Chinese dumplings), and steamed buns, are more popular in Northern China, some regions in particular like the provinces of Shandong, Henan and Shanxi. The regions of Northeast China (the provinces of Liaoning, Heilongjiang Shenyang) are known to keep the traditional heavy food patterns with energy-dense products [515] because of long, dry and cold winter. Due to the heavy dietary intakes, the traditional northern pattern was reported to be associated with higher prevalence of hypertension and blood pressure [515].

Conversely, in the Southern and Eastern regions of China, grain processed foods, such as rice and rice processed foods (rice noodle), are more popular than wheat flour processed foods as a consequence to the warm temperatures and lots of rain, high humidity. Because of variety of dietary patterns and non-greasy foods, the traditional southern pattern was reported to be related to the lower prevalence of hypertension and blood pressure [515]. However, the nutrition and lifestyle transition toward Western dietary patterns with sedentary lifestyle occurred in some well-developed regions such as east coast (Jiangsu province, Shanghai, Zhejiang province), due to the economic growth and Western culture influences [515]. Therefore, a new dietary pattern- the Western dietary pattern has been observed in the urban areas and is becoming more and more popular. In addition, the traditional cuisines in the

Jiangsu province and municipality of Shanghai have a little sweet taste as people get used to adding a little sugar or sweet additive. In the Southern China, variety is the key to this tradition. vinegar is one of the most welcome seasoning is part of that regions such as Guangxi province [515]. Furthermore, chili is one favorite flavorings in southwestern China because of the climate, geography and dietary culture

3.5. Measurements

3.5.1. Anthropometric measurement

Anthropometry has a widespread and important place in nutritional assessment and clinical studies [184], because it gives insight into the nutritional and health status of individuals along with predisposition of primary risk factors. In order to assess nutritional and health status, height and weight are mostly often selected to use, because these measures are quick, simple cheap and require limited training. In addition, BMI, calculated based on height and weight, is a well-known predictor of OB in the general population [319]. However, BMI is not an accurate indicator of body composition [319]. Body composition including weight, height, WC, BF% and HC were collected and used for the different populations in this thesis. All the measurements were following a strict protocol and technical procedures. Therefore, more comprehensive measures including circumferences and BF% are better and stronger predictor of abdominal OB and OB-related health risks [92;343]. Hence, more than one predictor were used in this thesis in order to find more precise associations between dietary protein and fiber intakes and body composition. The details of the anthropometric measurement have been described in each chapter.

As discussed in the part about "*Misreporting",* the self-reported weight and height of the Belgian population may result in numerous missing values or bias in specific subgroups [213]. However, the quality of self-reported weight, height and calculated BMI were checked with the comparison to the measured WC. In case of significant discrepancies between self-reported data and measured data of WC, those data were excluded in the final data analysis. In the meantime, the comparison of self-reported anthropometric data was checked between each interview record. The quality of anthropometric values was considered as acceptable, which indicates that no dominating error would influence the precise associations.

The anthropometric data of weight and height for the preschoolers was not valid because of parental reporting with low quality data of non-response or incomplete data, from low SES in particular. These results have been reported elsewhere [227]. Hence, the data was excluded in the final data analysis for the association with OW and OB.

The cut-off values of all the anthropometric data in this thesis were used following standard guidelines, Although the cut-off values of the Chinese population are different for those used in the Belgian population and European adolescents, scientific evidence suggests that Asian populations have different associations between BMI, BF% and health risks than do European populations [532]. WHO reported that current WHO cut-off values (normal-weight:18.5–24.9 kg/m^2, OW: $\geq$ 25.0 kg/m^2 and OB: $\geq$ 30.0 kg/m^2) do not provide an adequate basis for taking action on risks related to OW and OB in many Asian populations, but are matching the characteristics of European populations [532]. Lower than standard cut-off points are strongly recommended for Asian population as well [532]. The group of the China Obesity Task Force made a guideline of cut-off points for OW and OB considering the characteristics of the Chinese population, which was used in the Chinese study described in the *Chapter 6.* Likewise, the cut-off points of WC for school-aged children and adults were also following the Chinese recommendations. Therefore, given the almost accurate self-reported and strictly measured weight and height of the Belgian population and European adolescents, it is concluded that the minor errors couldn't have influenced exposure of health outcome (e.g. OB), although minimal errors couldn't be avoided.

3.5.2. Biomarkers

Biomarkers were only collected among European adolescents in the HELENA-CSS. As known that anthropometric measurements such as weight, height and WC are very popularly and widely used in epidemiological, clinical and population based studies because of easy data collection and less expensive measures [184]. However, anthropometric data are not strong health indicators compared to biomarkers. Biomarkers are commonly and importantly used as health indicators for health status in epidemiological, pre-clinical and intervention studies as they play a critical role in helping in early diagnosis, disease prevention [350]. For example, the values of blood cholesterol are widely known as risk indicators for coronary and vascular diseases, and the levels of blood glucose, insulin and hemoglobin are indicators for pre-T2D and T2D. However, it is expensive and difficult to collect blood sample in a large scale population-based survey.

Blood samples in the HELENA-CSS were collected at hospitals by a medical doctor following regulations and measured by advanced technology in lab. So the values of the blood samples were valid and accurate to be representative for participants' health status. The details of biomarkers have been described in Chapter 2 and Chapter 4. Whereas, blood samples were collected in a subsample of the total sample participating in the HELENA-CSS. Therefore, the small sample size of serum biomarkers may be a potential influencing factor leading to weak associations between dietary protein and fiber intakes and serum biomarkers. However, the strong statistics power prove the valid associations.

4. DIETARY RECOMMENDATIONS

As known, consuming a healthy diet with a variety of food items, containing high amounts of plant based foods and few amounts of foods from animal sources and processed foods, helps prevent NCDs, including diabetes, heart disease, stroke and cancer.

The results reveal that total protein intakes among different sub-groups of all the populations in the studies were adequate based on the comparison with different recommendation values, although total protein intake in the Chinese population only reached the international level and was below the Chinese guidelines, as described in each chapter. However, plant- based protein intakes are much lower in the European populations than proteins from animal sources. Only the Chinese populations consumed more plant- derived proteins than animal-derived proteins (*Chapter 6*). In addition, DF intakes based on the findings in this thesis were shown insufficient in all the sub-groups of all the populations under study according to the national and international recommendations, even though the Chinese population consumed plant-based diets. Moreover, the increased consumption of energy-dense, low-nutritious processed food, rapid urbanization and changing lifestyles have led to a shift in dietary patterns [465]. Therefore, many people in Europe and China are consuming high intakes of convenience foods, such as hamburgers and semi-prepared foods, which are rich in energy, saturated fats, trans fats and sodium. Building a positive and healthy nutrition environment can have the most sustainable impact on health outcome.

Some common regulations and strategies need to be taken into account to create and maintain a healthy food environment for both the European and Chinese populations. Policy intervention can be very efficient for addressing nutrition imbalance and popularizing the consumption of plant – based foods.

- Public education and the impact of mass media

The local government needs to advocate the series of public education for the association between nutrition knowledge and health outcome through schools, workplaces, community and mass media. Evidence shows nutrition and health knowledge is strongly related to the intake of fruits, vegetables and fat, and physical activity [314]. The public education programs should promote an understanding of the basic principles of proper portion size, healthy eating and lifestyle, which can be established regularly. Each education program can pre-define the target participants, age group, local culture, health topic and health problem. For example, at workplaces/communities, one specific health topic/disease can be set up at each public education program. Nutritionists/ medical doctors/researchers can educate these subjects to improve participant's right nutrition and health knowledge, correct lifestyle/dietary behaviors and change the attitudes of food choices and food shopping.

As known, mass media plays an important role in affecting individuals' food choices. Mass media can influence individuals' subjective choice (negative or positive guideline), which may result in the different expressing way of behaviors, women and children in particular. Hence, public mass media need to be set up as an education tool providing positive knowledge and information, while those unhealthy messages should be discouraged.

- Increase the availability of food markets

Apparently, due to the higher cost for high quality and healthy foods, families with a low SES purchase small varieties of foods and low quality of foods [414]. Increasing the number of food markets (e.g. supermarket, grocery and food open market) in the urban and rural communities can benefit to lower and moderate- income residents/families to more accesses to foods, because it can result in decreasing the food price due to the severe competition. In addition, increasing the availability and convenience are those that provide more variety of healthy food choices.

- Promote greater access to healthy, affordable foods and beverages in institutions, restaurants, schools, work place and industries

Generally, people consume more calories and fat when eating meals away from home. There is also apparent that foods of lower-nutrient density are more available in many schools than home-made foods. In some regions/cities/provinces of China, government has made effective use of **school feeding programs** to improve the food intake of school-aged children. However, rising food prices can affect the **school feeding programs** negatively. Although food price is not the main driver of unhealthy dietary behavior, they do affect nutrition outcomes through their impact on purchasing behavior due to government budget, which also happens to household purchasing relating to real household income.

Government can establish an effective policy to support **food supply chain programs** including production chain and supply chain. The **food supply chain programs** can help schools, supermarkets, working place gain foods directly from farms and food industries, which can get lower food price and avoid for the impact of inflation.

- Promote pricing strategies and effective pricing policy

Evidence suggests that price policies applied to food can influence food choices and dietary behaviors [170]. Pricing policies, especially in the form of reduced prices for healthy foods, are to influence purchase decision-making, which is more effective for promoting healthy eating behaviors [170]. Pricing policies can include encouraging the sale or consumption of healthy food items or discouraging the sale or consumption of non-healthy foods and high-energy dense foods. That can be formulated by reducing tariffs and other taxes for healthy food items, and increasing prices and taxes for non-healthy foods and high-energy dense foods.

- Increase opportunities to purchase local foods.

The recommended intakes of fruits, vegetables, legumes decreased not only in the European population, but also the Chinese population. Beside the above mentioned **food supply chain programs** to promote healthy dietary patterns, adopt policies that promote agricultural production productively on vacant and existing species, thus it is a mean for improvement of healthy food access, promoting agricultural entrepreneurism.

Preschoolers and school-age children

Preschoolers (3-6 years)

Parental attitudes, lifestyle and dietary behavior must certainly affect their children directly/ indirectly through the foods purchased and served in the household influencing the children's exposure and their habits and preferences [78]. Hence, **school-based nutrition education programs** involving parents' participation can be effective for establishment of children's early healthy food knowledge. However, pressuring children to eat and restricting access to specific foods are not recommended because it often leads to negative food behaviors such as overeating, dislikes, and interest in forbidden items [185]. In addition, school teacher can help parents supervise children's diets to consume a healthful diet and thus provide consistent improvement of diet quality at school and home.

School-aged children and adolescents (7-18 years)

As children grow up, various sources of food and influences on eating behavior increase. Many children are home alone and prepare their own meals because of parental work schedules. In addition, many meals and snacks are obtained away from home, often without supervision. Younger children should have more supervision from parents and teachers as they need more attention compared to the older. Older children like adolescents would have their own preference for self-selected foods, usually, which are sweetened beverages, snacks and fast foods contributing to excess consumption of calories with fewer nutrients.

As known, ***adolescents***, including pre- adolescents, are undergoing a special dynamic period which is responsible for growth of hormone, development of body weight and body shape, and the changes of behavior and lifestyle. So extra caloric and global nutrition are needed due to pubertal growth stimulating appetite. Currently, extra energy intake with an increasingly sedentary lifestyle, because of a decline of outdoor physical activity, results in high risk of central OB. While, because of the special period, adolescents, especially for girls, pay more attention to physical appearance. Therefore, skipping breakfast and insufficient consumption are very common during adolescence in order to keep body shape. Besides the recommendation for the preschoolers and parental positive modeling as an important role in establishing children's food choices, food preference and dietary behavior [360], **school-based nutrition education programs** need to include **school-based self-esteem intervention,** which could improve the understanding of body image and eating attitudes and behaviors of young male and female adolescents. So they will have confidence for the body satisfaction.

For the ***Chinese children***, because of Chinese special policy: China's One Child Policy and the rapid economic growth, which result in a sharp increase in the number of OW and obese children, boys in particular. As the majority of children today have grown up in one-child families, they with special name 'Little Emperor' have become a spoiled generation, which eventually result in superior nutrition and health care. Parents involved in the **school-based nutrition education program** need to be educated the essentially weak point of over-protection and over-feeding.

Adults

1. Eat a variety of foods

People are strongly recommended to consume a variety of foods, which will supply sufficient calories, protein, essential fatty acids, carbohydrates, vitamins, minerals, and fiber needed for good health.

As based on the results in the thesis, adults, older people, the European peer in particular, have a higher risk of development for OW and OB, and the consequences of chronic diseases than the young adults and children. The various dietary patterns can achieve the positive for health outcome. Therefore, each individual needs to consume daily foods in variety, balance, and moderation, with emphasizing an intake of healthy food patterns, such fruits, vegetables, cereals and legumes, but lower energy-dense foods.

2. Balance food intake with physical activity and maintain or reduce weight

As discussed before, higher prevalence of OW and OB were found in older adults (*Chapter 4. and Chapter 6*), besides plant-based foods recommended, moderate outdoor physical activity is necessary to maintain long-term healthy weight, improve blood lipid levels and blood pressure, which can protect against the consequences of OB including CVD, T2D, stoke and some cancers.

3. Choose a diet low in fat, saturated fatty acids, and cholesterol, emphasize on the consumption of plenty of vegetables, fruits, legume and whole-grain products.

Based on the results from *Chapter 6.* the entire Chinese population still followed the plant-based dietary behavior, but nutrition transition has been observed, the big cities and urban areas in particular. The old generation, post-menopause women in particular, need to avoid low nutrition energy-dense foods. Eating plenty of vegetables, fruits, legumes and whole-grain products can help maximize a reduction in plasma total and LDL-C levels. For example, people consuming food containing 6.25 g soy protein per serving or 25 g of soy protein per day can reduce the risk of heart diseases by reducing blood cholesterol levels [166].

4. Choose a diet in moderation of sugar, sodium

Plant-based foods are rich in protein and fiber. Although DF and sugar both belong to carbohydrates, and DF is strongly recommended to consume daily due to health benefits. However, refined carbohydrates will increase energy intake. In addition, added sugar has no nutritional value and does not serve any physiological function in human body. On the country, extra sugar intake can cause health problems, such as weight gain, storage of fat mass and an increase in blood TC,TG and blood glucose levels.

Sodium plays a role in fluid balance in the human body. The more intake of sodium is related to the higher risk of hypertension, which may in turn increase the risk of heart disease, stroke, and kidney damage. Higher amount of salt in the diets comes from meat products and processed foods during food processing or during food preparation. While lower amount of salt in the diets occur naturally in fresh foods, like fruits, vegetables and legumes. Eating less salt from fresh foods and more potassium-rich foods with positive lifestyle changes may assist preventing or delaying to get high blood pressure and lowering high blood pressure.

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CHAPTER 8

GENERAL CONCLUSION

OB is one of the most serious public health concerns worldwide, as it increases the risk of many related chronic diseases including hypertension, CHD, T2D and some cancers. Due to the nutrition transition together with lifestyle changes, epidemiologic transition shifting from a pattern of high prevalence of infectious diseases related to undernutrition and environmental sanitation towards a pattern of high prevalence OB and NCDs related to over-nutrition is obviously observed in the developing countries. The high prevalence of OB and the related diseases is often found in urban areas now, which is the reverse of what is seen in the developed countries.

Diet plays an important role in the development of OB and in people's health status. Evidence shows that plant-based foods, rich in dietary protein and fiber, like fruits, vegetables, whole grains and legumes as important components of traditional diets in Asian regions might contribute to the lower risk of OB and other chronic diseases in Asian regions. This thesis focused on the evaluation of dietary protein and fiber intakes in four independent large-scale populations, including Flemish preschoolers, the Belgian population, European adolescents and the Chinese population, and on associations of protein and fiber intakes with OB, cardio-metabolic indicators, SES and lifestyle factors.

This thesis revealed that plant foods were inadequate for all the populations included in this thesis based on the ratio of animal and plant proteins, fiber intakes and their main contributors. Plant-based foods were reported the main contributors to the daily dietary intakes in the Chinese dietary pattern, whereas animal-based foods, considered as the typical Western dietary pattern, were the main sources contributing to the intakes in the rest of the study populations. Because of the nutrition transition and time period of the studies included in this thesis, the Chinese population in the urban areas and relatively rich regions now consume daily intakes close to the Western dietary pattern rich in animal food sources based on the literature. This is consistent with the findings in The China Health and Nutrition Survey (Chapter 6), which shows the rich region (SM) consumed approximately 50% total protein from animal sources. However, as the nutrition transition already happened several decades ago in Europe, the dietary pattern in the European population is currently quite stable and very likely comparable with that a decade ago.

Parental SES and lifestyle play the major role in the establishment and maintenance of healthy dietary behaviors and lifestyle among children and adolescents. The parents' positive health nutrition knowledge, influence and involvements such as eating breakfast, consuming more fresh fruits and vegetables with less soft drinks, providing appropriate portion sizes, healthy eating habits and having right food choice and food decision are very important and necessary. Besides healthy eating habits, a less sedentary lifestyle and more outdoor physical activity are important positive factors as well. The study of Flemish preschoolers demonstrated that high-nutritious foods and quality foods were associated with parental SES and lifestyle of both preschoolers and parents.

This thesis revealed that the association between indicators of OW and OB and animal/plant proteins and DF varied according to the characteristics of the target populations. In general, compared to the Western dietary pattern, plant-based foods, the traditional Chinese dietary pattern rich in plant protein and DF, is inversely associated with OW and OB, and the related chronic diseases via reducing body weight, building body composition and improving blood lipid profile. While foods from animal origin are opposite associated with these indicators.

The consumption of diets rich in legumes, vegetables and other plant food sources, on one hand, was very low in European populations; on the other hand, disappeared significantly in urban areas of China. Therefore, some regulations and strategies need to be taken into account for keeping a healthy and balanced diet in European and Chinese populations including increasing the availability of food markets, pricing strategies and public education.

Moreover, the effect of different dietary patterns (e.g. animal based diet vs. plant based diet; the Western normal diet vs. the Chinese medical diet) on gene expression for prevention of OB and chronic diseases should be carried out for future longitudinal studies.

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Summary

OB is a serious epidemical concern worldwide with dramatically rocketing OB prevalence over the past few decades. OB and its related consequences of various chronic diseases, including CVD, T2D, obstructive sleep apnea, certain types of cancer and osteoarthritis have been concerned for threatening human's health status and influencing quality of life. With decreasing consumption of plant foods and increasing consumption of foods-derived from animal sources not only in Western countries, but also in the developing countries, nutrition disorders have been considered to result in higher risks of OB and OB related chronic diseases.

Dietary protein and fiber are evidently considered as important macronutrients for improvement of human's health status and disease prevention. Dietary proteins, made of strings of amino acids forming chains known peptides, are essential nutrients for the human body providing energy and building body tissue and bone mass. As energy sources, proteins contain 4 kcal per gram like carbohydrates. Concerning the food sources, dietary proteins can be divided into two types: animal proteins and plant proteins. ***Animal proteins*** mainly comes from animal sources such as meat, dairy, fish and egg. While ***plant proteins*** are derived from vegetables, fruits, grains, nuts and legumes.

Dietary fiber, coming from plant foods and made up of NSP, such as cellulose, dextrins, inulin, lignin, chitins, pectins, beta-glucans and oligosaccharides, are divided into ***WSF*** and ***WIF***, which contribute differently to human's health. Soluble fiber dissolves in water. ***WSF*** is readily fermented in the colon producing gases and physiologically active byproducts such as short-chain fatty acid and being prebiotic and viscous, which causes a longer-lasting satiety and lowers the glycemic index of foods. ***WSF*** tend to slow the movement of foods through the system, which may reduce cholesterol absorption. Due to its anti-nutritive properties and non-digestibility, ***WIF*** can increase the bulkiness of stool and faecal mass, thereby shortening transit time in the system.

The overall objective of this thesis is to study and compare the difference of dietary intakes of protein and fiber in the traditional Chinese dietary pattern and the Western dietary pattern and to investigate the associations with OB and OB-related consequences due to the difference of dietary intakes caused by cultural and dietary behaviors. **The primary objective** of this thesis is to evaluate the consumption of total dietary protein and fiber in four populations and to determine the main contributors to total dietary protein and dietary fiber intakes in both dietary patterns. Based on the results of each study, the levels of dietary protein and fiber intakes were evaluated by the national or international recommendations/guidelines. **The secondary objective** is to examine the associations between the consumptions of total dietary protein and DF, or their main contributors, and indicators (anthropometry and biomarkers) of OB and chronic diseases. Because of the lack of valid anthropometric data in Flemish preschoolers, the investigation on the association of intakes with indicators of OB was substituted by the factors of SES and lifestyle, which are important for the establishment of dietary habits and the development of body composition in the early childhood. Hence, **the third objective of this thesis** is to examine the association between animal and plant proteins, total DF and food group-specific protein/ fiber intakes and SES, lifestyle-related factors in Flemish preschoolers.

The data included in this thesis was collected from four independent research surveys (FPDS, BNFCS, HELENA-CSS and CHNS). Therefore, the target populations in this thesis are composed of four independent groups: 1. Flemish preschoolers aged from 2.5 to 6.5 years (661 participants in the final analysis, 51% males); 2. Belgian population aged ≥ 15 years (3083 participants in the final analysis, 50% males); 3. European adolescents, recruited from 8 European cities, aged 12.5-17.5 years (1804 participants in the final analysis, 47% males); 4. Chinese population aged ≥3 years (9720 participants, 49% males).

All the dietary intakes with the exception for the Flemish preschool dietary survey were collected via two/three 24-h dietary recalls. Dietary intake derived from the Flemish preschool dietary survey was collected using 3-d EDR completed by parents. Dietary total, animal and plant proteins, DF, WSF and WIF intakes of the European populations in this thesis were estimated based on the Belgian NUBEL, the Dutch NEVO and the USDA food composition databases. The intakes of protein and fibers in the Chinese population were estimated using the Chinese Food Composition Table. Each survey followed its own standard research design, methodology and protocol, which has been described in the methodology section in each chapters and *Chapter 2.*

To reach the second and third objectives, anthropometric data, including BMI and WC were collected for investigating the associations with protein and fibre intakes. In addition, biomarkers were collected and used to investigate associations between intakes and physiological indicators of OB and chronic diseases in European adolescents.

The results of seven research studies derived from four independent research surveys reveal that total protein intakes were adequate for all these four populations based on the recommendation proposed by WHO/FAO/United Nations (i.e. 10–15% of the total energy intake). Meat and meat products contributed most to total and animal protein intakes for all the target populations with the exception for the Chinese population, where 74% of the proteins were from vegetable origin. Concerning the plant proteins, cereals and cereal products contributed most to plant protein intake in all populations. In contrast with the adequate protein intakes, was DF intake found being inadequate in all four populations. Cereals and cereal products were the largest contributor to dietary fiber intake in all the target populations with the exception for the Chinese population. Vegetables were the largest contributors to dietary fiber in the Chinese population.

What is the importance of **the primary objective** ?

Although the nutrition transition has been shifted towards Western dietary pattern gradually due to the rapid economic growth and urbanization in China, Chinese, apparently, still kept the traditional Chinese plant-based diets (cereals, vegetables and legumes). However, compared to Chinese diets, European diets were rich in animal sources (meat and meat products, and dairy products) with low intakes of vegetables and fruits, which led to the epidemical health consequences. In addition, food preparation is a key factor leading to the difference in intakes. The diets and dietary culture are various because of the demography. The highest energy and, total and animal protein intakes, like in Western diets, were found in the Chinese " sweet but less heavy meals" region, which could be influenced by economic growth. While energy-adjusted dietary fiber was highest in "flour (pasta)-rich meals". Therefore, the dietary habits are related to lifestyle and economic factors.

Regarding the associations of OB and cardio-metabolic indicators with dietary protein and fiber, which is answering to **the secondary objective** of this thesis, much higher prevalence of Belgian adults were defined OW and OB, compared to the Chinese adults, the old generation in particular. Evidence shows that a healthy diet rich in grains, potatoes and tubers, legumes, vegetables and fruits can protect against chronic diseases. Plant sources still dominated Chinese daily diets. The findings show moderate total and plant protein intakes may play a role in building body composition and preventing OB and its related chronic diseases in the Belgian population, European adolescents and Chinese adults. The findings reveal that total dietary fiber intake was inversely associated with BMI, body composition and blood lipid profiles in the Belgian population and European adolescents. Regarding the type of dietary fiber, it was found that WSF can protect against insulin resistance and its concomitant diseases.

Biomarkers were only used to evaluate the associations in European adolescents of the HELENA study. However, the results were not as expected as only few associations were found, which was similar in Chinese children. It is generally known that pre-puberty and puberty are critical periods in life characterised by more independent responsibility about his/her own dietary intakes, changes in dietary behaviors, development of the body composition and, hormone changes and regulation.

Regarding **the third objective** of this thesis, it has shown that parental SES was associated with preschooler's protein and fiber intakes, maternal education and employment in particular. Generally, high intakes derived from vegetables and fruits, on one hand, were observed in the children with relatively high educated parents; on the other hand, preschoolers with low and moderate PA had lower animal and plant protein intakes compared to preschoolers with high PA levels. Evidence shows parental influence as the most important factor impacting on maintaining body weight and the development of body composition in children's early life, affecting health consequences in the adult's life, because of dietary intake and lifestyle.

The overall objective of this thesis was to compare differences between the Western dietary pattern and the Chinese dietary pattern, and their association with OB and its concomitant co-morbidities. As presented before, approximately 74% of food intakes were from vegetable origin in the Chinese population, whereas the ratio of animal-to-plant source approximates 2:1 in European diets presented in this thesis. The huge difference may result in higher risk of developing OB and its consequences in the European populations. Moreover, it can be concluded that a various dietary pattern rich in plant sources in human diets complying with a positive lifestyle and nutrition knowledge can provide essential daily nutrients and energy, and play a role in preventing OB and the consequence of OB-related co-morbidities.

What can we do to build and keep a healthy and balanced diet?

Some regulations and strategies might be adjusted to play an important role in creating a healthy purchasing environment and building nutrition and health knowledge. The adjustments may focus on 1) public education to improve nutrition and health knowledge and reducing the bias of mass media; 2) creating more access to healthy and fresh foods in the local food market, schools, workplaces and industries; 3) promoting pricing strategies and effective pricing policy in order to decrease price for fresh foods and increase price for low nutrition, energy-dense foods.

This thesis didn't investigate the causality of the observed associations, however, it shows the values of the huge difference in dietary patterns between Chinese and European populations influencing several health outcomes. Therefore, **future research** should focus on longitudinal studies observing time trends in dietary patterns affecting the Chinese and Western dietary intakes. Thus, any causal relationships between protein and fibre intakes should be further investigated in longitudinal study designs.

Moreover, time trends in impacts on genomics should be taken into account as a research objective for future longitudinal studies, as it is related to promote and deteriorate health status and health consequences. These studies can provide valuable information concerning the effect of foods/ dietary patterns/nutrients on gene expression for prevention of OB and chronic diseases. Therefore, personalized dietary patterns can be advised to improve nutritional quality and health outcomes.

Samenvatting

Obesitas is een ernstig epidemisch en wereldwijd probleem met een explosieve obesitas prevalentie in de afgelopen decennia. Obesitas en de bijbehorende gevolgen van verschillende chronische ziekten, zoals hart- en vaatziekten, type 2-diabetes, obstructieve slaapapneu, bepaalde vormen van kanker en osteoarthritis vormen een bedreiging voor de menselijke gezondheid en beïnvloeden de levenskwaliteit. Met een afnemende consumptie van plantaardig voedsel en de toenemende consumptie van voedingsmiddelen afkomstig van dierlijke bronnen, is vastgesteld dat voedingsstoornissen resulteren in een hoger risico op obesitas en obesitas gerelateerde chronische ziekten, en dit niet alleen in de westerse landen, maar ook in de ontwikkelingslanden.

Voedingseiwitten en vezels worden uiteraard beschouwd als belangrijke macronutriënten ter verbetering van de menselijke gezondheid en in het kader van ziektepreventie. Voedingseiwitten, opgebouwd uit aminozuurketens zijn essentiële voedingsstoffen voor het menselijk lichaam. Eiwitten leveren energie, namelijk 4 kcal per gram. Ze zijn ook bouwstenen voor het lichaamsweefsel en botmassa. De eiwitten kunnen onderverdeeld worden in twee types: dierlijke en plantaardige eiwitten. ***Dierlijke eiwitten*** zijn voornamelijk terug te vinden in dierlijke bronnen zoals vlees, zuivel, vis en eieren. ***Plantaardige eiwitten*** daarentegen worden verkregen uit groenten, vruchten, granen, noten en peulvruchten.

Voedingsvezels, afkomstig uit plantaardige voedingsmiddelen en uit niet-zetmeelhoudende polysachariden, zoals cellulose, dextrine, inuline, lignine, chitine, pectine, bèta-glucanen en oligosachariden, worden onderverdeeld in ***fermenteerbare voedingsvezels*** en ***niet-fermenteerbare voedingsvezels***, en dragen op een verschillende manier bij tot de gezondheid van de mens. Oplosbare vezels lossen op in water. ***Fermenteerbare voedingsvezels*** fermenteren gemakkelijk in de dikke darm waarbij gassen en fysiologisch actieve bijproducten aangemaakt worden, zoals korte-keten vetzuren, met een prebiotische en fermenterende werking. Deze fermenteerbare voedingsvezels zorgen voor een langduriger verzadigingsgevoel en verlagen de glycemische index van voedingsstoffen. Zij hebben de neiging om de beweging van voedsel doorheen het systeem te vertragen, waardoor de opname van cholesterol vermindert. Door zijn antinutritieve en onverteerbare eigenschappen kunnen ***niet-fermenteerbare voedingsvezels*** de fecale massa doen toenemen, waardoor de transittijd in het systeem verkort.

De algemene doelstelling van dit proefschrift is het bestuderen van de eiwit- en vezelinname zoals geregistreerd in het "Chinees" én het "Westers" voedingspatroon, het vergelijken van de verschillen omwille van andere culturele gewoonten en voedingsgedrag én vervolgens het bestuderen van associaties met obesitas en obesitas gerelateerde gevolgen. **De primaire doelstelling** van dit proefschrift is het evalueren van de totale consumptie van eiwitten en vezels binnen vier bevolkingsgroepen en het definiëren van de belangrijkste eiwit- en vezelbijdragers, in beide voedingspatronen. Op basis van de resultaten van elk onderzoek werd het innamegehalte van eiwitten en vezels geëvalueerd door nationale of internationale aanbevelingen/richtlijnen. **De tweede doelstelling** betreft het onderzoeken van associaties tussen de totale consumptie van eiwitten en vezels of van de belangrijkste eiwit- en vezelbijdragers én de indicatoren (antropometrie en biomerkers) van obesitas en chronische ziekten. Vanwege het gebrek aan geldige antropometrische gegevens bij Vlaamse kleuters, werd het onderzoek naar de associatie van inname met obesitasindicatoren vervangen door factoren met betrekking tot de sociaaleconomische status en levensstijl. Deze factoren zijn, reeds van in de vroege kindertijd, belangrijk voor de grondlegging/fundering van voedingsgewoonten en de ontwikkeling van de lichaamssamenstelling. Vandaar de **derde doelstelling van dit proefschrift**, met name het onderzoek naar de associatie tussen dierlijke en plantaardige eiwitten, de totale hoeveelheid vezels, de voedingsmiddelengroepspecifieke eiwit- en vezelinname, én de sociaaleconomische status en levensstijlfactoren bij Vlaamse kleuters.

De gegevens in dit proefschrift werden verzameld uit vier onafhankelijke onderzoeken (FPDS, BNFCS, HELENA-CSS en CHNS). Daarom werden de doelgroepen in dit proefschrift samengesteld uit vier onafhankelijke groepen: 1. Vlaamse kleuters van 2,5-6,5 jaar (661 deelnemers in de finale analyse, 51% mannen); 2. Belgische bevolking van ≥ 15 jaar (3083 deelnemers in de finale analyse, 50% mannen); 3. Europese jongeren uit 8 Europese steden, in de leeftijd van 12,5-17,5 jaar (1804 deelnemers in de finale analyse, 47% mannen); 4. Chinese bevolking van ≥ 3 jaar (9720 deelnemers, 49% mannen).

De totale voedingsinname, met uitzondering van het voedingsonderzoek bij de Vlaamse kleuters, werd verzameld via twee/drie 24-u voedingsbevragingen (Dietary Recall). De inname afkomstig van het voedingsonderzoek in de Vlaamse kleuterscholen werd verzameld met behulp van eetdagboekjes (3 dagen, Estimated Dietary Record), ingevuld door de ouders. De totale inname, dierlijke en plantaardige eiwitten, vezels, fermenteerbare en niet-fermenteerbare voedingsvezels bij de westerse bevolking, werd in dit proefschrift geschat op basis van de Belgische NUBEL, de Nederlandse NEVO en de USDA voedingsmiddelentabellen. De inname van eiwitten en vezels bij de Chinese bevolking werd geschat met behulp van de Chinese voedingsmiddelentabel. Elk onderzoek volgde zijn eigen standaard onderzoeksdesign, methodologie en protocol, zoals beschreven in het deel methodologie in elk hoofdstuk en *Hoofdstuk 2*.

Om de tweede en derde doelstelling te bereiken werden antropometrische gegevens, waaronder BMI en tailleomtrek, verzameld voor onderzoek naar de associaties met eiwit- en vezelinname. Daarnaast werden biomarkers verzameld en gebruikt om associaties te onderzoeken tussen inname en fysiologische indicatoren van obesitas en chronische ziekten bij Europese adolescenten.

Uit de resultaten van zeven onderzoeksstudies, verkregen uit de vier onafhankelijke onderzoeken en gebaseerd op aanbevelingen van de Wereldgezondheidsorganisatie , de Voedsel- en landbouworganisatie van de Verenigde Naties (i.e. 10-15% van de totale energie-inname), blijkt dat de totale proteïneconsumptie voldoende adequaat was in alle vier populaties. Vlees en vleesproducten dragen het meest bij aan de totale en dierlijke eiwitinname voor alle doelgroepen, met uitzondering van de Chinese bevolking waar 74% van de eiwitinname afkomstig is van plantaardige voedingsmiddelen. Granen en graanproducten zijn de grootste leveranciers van plantaardige eiwitten, en dit bij alle populaties. In tegenstelling tot de adequate eiwitinname bleek de inname van voedingsvezels ontoereikend bij de vier populaties. Granen en graanproducten waren de grootste bijdragers aan voedingsvezels in alle doelgroepen, met uitzondering van de Chinese populatie. Binnen de Chinese populatie waren groenten de grootste aanbrengers van voedingsvezels.

Wat is het belang van de **primaire doelstelling** ?

Alhoewel het voedingspatroon geleidelijk is verschoven naar een meer westers patroon als gevolg van de snelle economische groei en de verstedelijking in China, houden de Chinezen zich blijkbaar toch nog steeds aan het traditioneel Chinees voedingspatroon met een voorkeur voor plantaardige voedingsbronnen. Vergeleken met het Chinees voedingspatroon, zijn de Europese voedingspatronen rijk aan dierlijke bronnen en een lage inname van groenten en fruit, met epidemische gevolgen voor de gezondheid. Zowel voedingsbereidingen als demografische factoren spelen hierbij een rol. De hoogste energie-inname en de inname van dierlijke voedingsmiddelen, zoals in het westers dieet, werden gevonden in de "zoete maar minder zware maaltijden" regio in China, als mogelijk gevolg van de economische groei. Daarom worden de voedingsgewoonten in verband gebracht met levensstijl en economische factoren.

Betreffende de associaties van obesitas en cardio-metabolische indicatoren met eiwitten en vezels, wat beantwoordt aan **de tweede doelstelling** van dit onderzoek, werd een veel hogere prevalentie van Belgische volwassenen met overgewicht en obesitas gedefinieerd, vergeleken met de Chinese volwassenen, en dan vooral de oude generatie. De Chinese dagelijkse voeding bestaat nog steeds uit voornamelijk plantaardige voedingsmiddelen. Het werd ook aangetoond dat een gezonde voeding rijk aan granen, aardappelen, knolgewassen, peulvruchten, groenten en fruit bescherming kan bieden tegen chronische ziekten. Uit bevindingen blijkt dat een gemiddelde totale inname van plantaardige eiwitten een rol kan spelen bij de opbouw van de lichaamssamenstelling en de preventie van obesitas en obesitas gerelateerde chronische ziekten. De bevindingen toonden aan dat de totale inname van vezels omgekeerd geassocieerd werd met BMI, lichaamssamenstelling en bloedlipiden profiel bij de Belgische bevolking en Europese adolescenten. Met betrekking tot het type voedingsvezel, bleek dat fermenteerbare voedingsvezels kunnen beschermen tegen insulineresistentie en bijkomende aandoeningen.

Biomarkers werden enkel gebruikt om de associaties bij Europese adolescenten uit de HELENA studie te evalueren. Echter, de resultaten waren niet zoals verwacht omdat er slechts weinig associaties werden gevonden, vergelijkbaar met die bij Chinese kinderen. Het is algemeen bekend dat prepuberteit en puberteit moeilijke periodes zijn in het leven die gekenmerkt worden door meer onafhankelijke verantwoordelijkheid over zijn/haar voedselinname, veranderingen in voedingsgedrag, ontwikkeling van de lichaamssamenstelling en hormonale veranderingen en regulatie.

De derde doelstelling van dit proefschrift heeft aangetoond dat de sociaaleconomische status van de ouders geassocieerd wordt met de inname van eiwitten en vezels bij kleuters, met de opvoeding door de moeder en werkgelegenheid in het bijzonder. In het algemeen werd enerzijds een hoge inname afkomstig uit groenten en fruit waargenomen bij kinderen met relatief hoog opgeleide ouders. Anderzijds hadden kleuters met weinig of een gemiddelde lichaamsbeweging een lagere inname van dierlijke en plantaardige eiwitten in vergelijking met kleuters met veel lichaamsbeweging. De invloed van de ouders wordt als de belangrijkste factor gezien voor het handhaven van het lichaamsgewicht en de ontwikkeling van de lichaamssamenstelling in de vroege kinderjaren, met mogelijke gevolgen voor de gezondheid in het leven van de volwassene, omwille van voedselopname en levensstijl.

De algemene doelstelling van dit proefschrift was om de verschillen tussen westerse en Chinese voedingsgewoonten te vergelijken en hun impact op obesitas en de daarmee gepaard gaande comorbiditeit. Zoals reeds eerder vermeld, is ongeveer 74% van de voedselinname van plantaardige oorsprong bij de Chinese bevolking, terwijl de verhouding van dierlijke versus plantaardige bronnen ongeveer 2:1 benadert in de Europese voeding. Het enorme verschil kan resulteren in een hoger risico op het ontwikkelen van obesitas en de gevolgen daarvan bij de Europese populaties. Bovendien kan worden geconcludeerd dat een gevarieerd voedingspatroon, rijk aan plantaardige bronnen, gecombineerd met een positieve levensstijl en kennis van voeding, kan voorzien in voldoende essentiële dagelijkse voedingsstoffen en energie en een rol kan spelen bij het voorkomen van obesitas en het gevolg van obesitas gerelateerde comorbiditeit.

Wat kunnen we doen om een gezond en evenwichtig voedingspatroon op te bouwen én te behouden?

Het aanpassen van sommige reguleringen en strategieën kunnen een impact betekenen bij het creëren van een gezond aankoopbeleid en kennis over voeding en gezondheid.

De focus kan hierbij gericht worden op 1) het geven van gezondheidsvoorlichting om de kennis rond voeding en gezondheid te verbeteren en om de consument te wapenen tegen verkeerde boodschappen via de media. 2) het verhogen van het aanbod van gezonde voedingsmiddelen in winkels, school- en werkomgeving. 3) het promoten van prijsstrategieën en effectief doorvoeren van prijsaanpassingen zoals het verlagen van de prijs van gezonde voedingsmiddelen én het verhogen van de prijs van voedingsmiddelen met een hoge energiedichtheid en lage voedingswaarde.

.Dit proefschrift onderzocht niet de causaliteit van de waargenomen associaties, maar toont de waarden van het enorme verschil in voedingspatronen, met beïnvloeding van verschillende gezondheidsresultaten, tussen Chinese en Europese populaties. Daarom moet **toekomstig onderzoek** zich richten op longitudinale studies waarin tijdtrends worden geobserveerd in voedingspatronen met een impact op de Chinese en westerse voedselinname. En daarom ook moet elke causale relatie tussen de inname van eiwitten en vezels verder worden onderzocht in longitudinale studiedesigns. Bovendien kan het onderzoek van "nutritional genomics", het bestuderen van relaties tussen het genoom, voeding en gezondheid, een meerwaarde betekenen in deze longitudinale studies. Waardevolle informatie betreffende het effect van voeding/voedingspatronen/nutriënten op genexpressie, in de preventie van obesitas en chronische ziekten kan hierbij bekomen worden. Gepersonaliseerde voedingspatronen kunnen aanbevolen worden om de kwaliteit van voeding en gezondheid te verbeteren.

Appendices

Appendix 1. Definition

1. What are preschoolers?

The term of preschool is relating to be the early years of childhood that precede the beginning of elementary school. The definition of age varies from one country to another country. Therefore, the preschool education is depending on the region. For example, in the United States, preschool precedes kindergarten and the normal primary school education, while in many European countries, preschool and kindergarten programs can be the same early childhood education programs.

Preschool children in Flanders of Belgium are defined as children between 3 to 6 years old based on the definition of Flemish FBDG, and children between 1 to 3 years old are considered as a toddler [507]. In this thesis, preschoolers were included between 2.5 and 6.5 years of age attending preschool in Flanders. In addition, Chinese preschoolers were included in the thesis between 3 and 6 years old based on the cut-off values of Chinese dietary guidelines [114].

2. What are adolescents?

Generally, adolescence describes the teenage years. This period is the transitional stage, including physical and psychological changes, from childhood to adulthood, which is called in term of <u>puberty or adolescence</u>. The period of adolescence represents as "critical period" and "dynamic period", which is responsible for physical growth and, the changes of behavior and lifestyle. European adolescents in the thesis were eligible between 12.5 and 17.5 years of age.

3. What are overweight and obesity?

WHO defined OW and OB as abnormal or excessive fat accumulation that may impair health [553]. OW can be considered a "pre-obesity" stage . BMI is a well-known and simple index of weight-for-height to describe the degree of a person's health status, which is commonly used to classify OW and OB in adults. It is defined as a formula: a person's weight (mass) in kilograms divided by the square of the person's height in meters (kg/m^2). Internationally, an adult who has a BMI between 25 kg/m^2 and 29.9 kg/m^2 is considered OW. While an adult who has a BMI of 30 kg/m^2 or higher is considered obese. Whereas, the cut-off values are recommended for Western/ European populations, which do not fit Asian population. Therefore, the cut-off values of OW and OB for Chinese adults were used based on Chinese

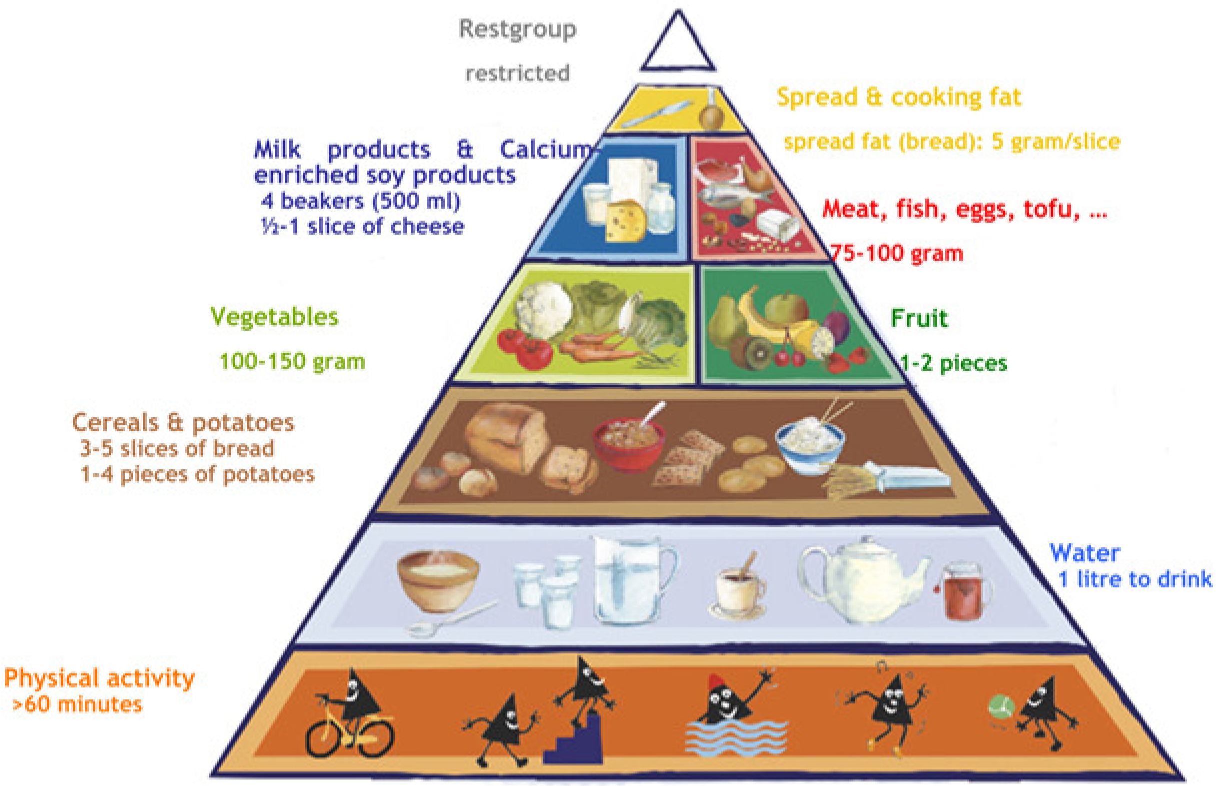
OB guidelines: BMI values of 18.5 to 23.9 kg/m^2, 24 to 27.9 kg/m^2 and above 28 kg/m^2 indicate normal weight, OW and OB, respectively.

The prevalence of child OB is increasing rapidly worldwide, the definition of child OB needs to be standard because BMI for adults doesn't taken into consideration with the age-sex/growth for children. Hence, the OW and OB for children and adolescents are defined respectively as being at or above the 85th and 95th percentile of BMI, which is suggested by the American Obesity Association. However, WHO expert committee recommended use of a weight-for-height z score for children aged < 10 years. Therefore, the Childhood Obesity Working Group of the IOTF developed a new international reference, age- and sex-specific BMI cutoffs, to define the degree of childhood and adolescent OW and OB, which is used worldwide [103].

Appendix 2. Flemish Food Based Dietary Guidelines (FBDG)

Food-based dietary guidelines are used to send simple messages on healthy eating and lifestyle in the public population. FBDG provides advice on foods, food groups and dietary patterns to provide the required nutrients to the general public to promote overall health and prevent chronic diseases.

In the Flanders, the food pyramid is used for food guides [508]. The food pyramid was expanded with the group 'physical activity' linked to a healthy lifestyle since 2004. The food pyramid is divided into seven levels, starting with physical activity at the bottom. Nine food groups are represented: cereals and potatoes; vegetables; fruits; meat, fish, eggs and meat alternatives; dairy and calcium-enriched products; oils and fatty products and sugary products; and unsweetened beverages such as water and tea; restgroup (imbalanced diet or energy-dense, low-nutritious foods).



Appendix 3. Total energy, total protein, animal and plant protein, and fiber intakes among Belgian population stratified by the age-region groups

	Flanders	SD	Brussels	SD	Walloon	SD	*P* [a]
Total energy (Kcal/day)							
15-18	2175.6	728.7	2019.0	745.0	2140.4	781.7	0.309
19-59	2080.0	886.5	1880.8	780.0	1989.8	727.2	0.112
60-74	1921.9	649.9	1809.9	705.2	1773.2	590.6	0.009
≥75	1749.4	577.8	1713.9	565.6	1672.4	579.2	0.286
Total protein (g/day)							
15-18	72.7	25.0	70.6	26.3	74.2	29.7	0.629
19-59	78.2	31.2	71.9	27.0	75.6	27.9	0.208
60-74	74.5	25.0	66.8	20.5	69.5	21.4	0.005
≥75	67.9	21.8	67.3	22.0	63.5	21.3	0.057
Total animal protein (g/day)							
15-18	44.9	20.6	44.5	21.6	48.5	25.0	0.117
19-59	50.5	25.2	47.1	21.8	52.1	23.4	0.327
60-74	49.2	20.5	45.0	17.5	47.5	17.6	0.204
≥75	44.8	17.5	47.7	19.6	42.9	16.7	0.160
Total plant protein (g/day)							
15-18	27.9	10.0	26.1	11.5	25.7	9.9	0.018
19-59	27.7	10.7	24.8	11.4	23.5	8.8	<0.001
60-74	25.3	9.1	21.8	8.5	22.0	8.5	<0.001
≥75	23.1	7.5	19.6	9.6	20.6	9.0	<0.001
Total fiber (g/day)							
15-18	17.5	6.6	15.4	6.4	14.3	5.7	<0.001
19-59	19.2	8.2	15.7	5.8	14.8	6.2	<0.001
60-74	20.7	8.4	16.3	6.1	16.9	6.8	<0.001
≥75	19.3	7.3	15.2	8.0	16.2	7.2	<0.001

SD, standard deviation

[a] *P* value for mean differences among the region groups (MANCOVA)

Appendix 4. Mean animal protein intakes (g/d) from main sources among Belgian population stratified by the age-region groups

Animal protein from main food sources	**Flanders**	**SD**	**Brussels**	**SD**	**Walloon**	**SD**	P^a
Dairy products							
15-18	13.4	10.8	13.2	10.9	13.5	9.2	0.640
19-59	13.9	10.5	12.6	9.1	14.6	11.1	0.480
60-74	12.0	9.2	11.3	10.6	12.4	8.8	0.212
≥75	11.6	8.6	13.9	9.1	10.8	8.2	0.049
Meat and meat products							
15-18	23.9	16.4	24.4	15.5	28.5	23.1	0.143
19-59	26.9	20.0	26.1	19.8	29.2	19.0	0.092
60-74	28.3	17.9	24.4	16.8	25.7	15.6	0.155
≥75	25.4	15.0	23.2	15.5	25.2	14.4	0.604
Fish and Shellfish							
15-18	3.0	6.1	3.6	6.2	2.6	5.5	0.635
19-59	5.1	9.0	5.2	8.2	4.0	7.3	0.357
60-74	5.3	8.4	6.3	8.9	5.3	8.7	0.317
≥75	4.4	8.4	6.4	8.0	3.9	6.6	0.029
Eggs and egg products (g)							
15-18	1.3	2.7	1.3	2.4	1.0	2.3	0.513
19-59	1.4	3.1	0.8	1.7	1.0	2.4	0.063
60-74	1.2	2.5	0.9	1.8	1.5	2.9	0.575
≥75	1.2	2.6	1.1	2.7	1.0	2.2	0.647

SD, standard deviation

[a] *P* value for mean differences among the region groups (MANCOVA)

Appendix 5. Mean plant protein intakes (g/d) from main sources stratified by the age-region groups

Plant protein from main food sources	Flanders	SD	Brussels	SD	Walloon	SD	*P*[a]
Potatoes and other tubers							
15-18	2.5	2.1	1.7	1.9	2.1	2.0	0.001
19-59	2.4	2.2	1.5	1.4	2.0	1.8	<0.001
60-74	3.1	2.1	1.9	1.8	2.9	2.1	<0.001
≥75	3.2	2.1	1.8	1.7	2.9	2.2	<0.001
Vegetables							
15-18	1.6	1.3	1.7	1.6	1.5	1.4	0.458
19-59	2.2	1.8	2.5	1.9	1.8	1.6	0.006
60-74	2.3	1.7	2.3	1.7	2.3	1.8	0.933
≥75	2.1	1.7	2.5	2.0	1.8	1.5	0.019
Legume							
15-18	0.044	0.324	0.249	0.864	0.112	0.653	0.004
19-59	0.185	1.3	0.534	1.9	0.147	0.769	0.004
60-74	0.126	0.852	0.349	1.4	0.163	0.723	0.084
≥75	0.122	0.763	0.132	0.730	0.110	0.702	0.908
Soya products							
15-18	0.260	1.8	0.031	0.230	0.078	0.807	0.009
19-59	0.310	1.4	0.042	0.253	0.157	1.4	0.006
60-74	0.129	0.980	0.254	0.953	0.201	0.983	0.553
≥75	0.091	0.883	0.156	0.679	0.100	0.838	0.374
Fruits, nuts and seeds							
15-18	1.0	2.0	1.3	2.8	0.7	1.6	<0.001
19-59	1.3	2.2	1.5	1.9	1.1	2.0	0.068
60-74	1.4	2.1	1.5	1.6	1.2	2.0	0.566
≥75	1.1	1.4	1.3	2.6	1.2	1.9	0.676
Cereals and cereal products							
15-18	15.9	8.1	15.7	9.4	14.9	7.4	0.237
19-59	16.0	8.8	13.9	9.4	13.6	7.0	0.001
60-74	13.1	6.7	11.2	6.5	11.3	6.4	<0.001
≥75	11.8	5.4	9.6	6.8	10.5	6.8	<0.001

SD, standard deviation
[a] *P* value for mean differences among the region groups (MANCOVA)

Appendix 6. Mean fiber intake (g/d) from main food groups among Belgian population, stratified by the age- region groups

Food sources	**Flanders**	**SD**	**Brussels**	**SD**	**Walloon**	**SD**	***P*** [a]
Potatoes and other tubers							
15-18	3.1	2.5	2.1	2.4	2.6	2.5	<0.001
19-59	3.0	2.8	1.8	1.9	2.5	2.3	<0.001
60-74	3.9	2.7	2.4	2.4	3.6	2.6	<0.001
≥75	4.1	2.7	2.2	2.1	3.7	2.9	<0.001
Vegetables							
15-18	2.1	1.7	2.0	1.6	1.8	1.7	0.022
19-59	2.8	2.3	2.8	1.9	2.2	2.0	<0.001
60-74	3.0	2.2	2.7	2.1	3.0	2.5	0.473
≥75	2.7	2.3	2.5	1.8	2.3	2.1	0.02
Legume							
15-18	0.051	0.374	0.181	0.617	0.103	0.593	0.004
19-59	0.187	1.3	0.478	1.7	0.141	0.859	0.004
60-74	0.154	1.1	0.262	1.0	0.152	0.681	0.086
≥75	0.135	0.877	0.090	0.486	0.090	0.552	0.904
Soya products							
15-18	0.260	1.8	0.031	0.230	0.078	0.807	0.009
19-59	0.305	1.4	0.042	0.253	0.157	1.4	0.005
60-74	0.124	0.915	0.254	1.0	0.201	1.0	0.568
≥75	0.091	0.883	0.156	0.679	0.100	0.838	0.374
Fruits, nuts and seeds							
15-18	2.1	2.5	2.8	3.0	1.5	2.1	<0.001
19-59	2.6	2.7	3.0	3.3	2.4	2.9	0.138
60-74	3.6	3.8	3.8	3.8	3.3	3.2	0.671
≥75	3.3	3.1	3.6	4.8	3.1	3.4	0.484
Cereals and cereal products							
15-18	6.7	3.9	5.4	3.0	4.9	2.7	<0.001
19-59	7.4	5.0	5.0	3.7	4.8	2.7	<0.001
60-74	6.7	3.9	4.4	2.6	4.4	2.9	<0.001
≥75	6.0	3.3	4.0	2.7	4.4	3.4	<0.001

SD, standard deviation

[a] *P* value for mean differences among the region groups (MANCOVA)

Appendix 7. Geographical map of study centers in HENELA-CSS

Appendix 8. The contributions (%) to total , animal and plant proteins takes in the adolescents participating HELENA study (2006-2007)

Food group	Total Protein			Animal protein			Plant protein		
	All	Males	Females	All	Males	Females	All	Males	Females
Beverages (including juices, excluding the rest group)	**7.2**	**7.8**	**6.3**	**1.4**	**1.6**	**1.2**	**16.2**	**17.7**	**14.0**
Water	0.3	0.4	0.2	0.2	0.3	0.1	0.3	0.4	0.2
Soups, bouillon	1.9	1.8	2.0	1.0	1.2	0.9	3.2	2.7	3.7
Coffee, tea	0.2	0.1	0.2	0.1	0.0	0.1	0.3	0.3	0.3
Fruit and vegetable juices	1.2	1.1	1.2	0.0	0.0	0.0	3.0	2.9	3.1
Carbonated/soft/isotonic drinks including non-alcoholic wine, non-alcoholic beer	3.6	4.3	2.6	0.0	0.0	0.0	9.3	11.2	6.7
Beer	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.2	0.1
Wine and cider	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other alcoholic beverages	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bread and cereals	**10.7**	**10.8**	**10.8**	**2.1**	**2.0**	**2.2**	**24.1**	**24.7**	**24.0**
Bread and rolls	9.5	9.6	9.7	2.1	2.0	2.2	21.2	21.6	21.3
Breakfast cereals	1.1	1.2	1.1	0.0	0.0	0.0	2.9	3.2	2.8
Potatoes and grains	**8.7**	**8.6**	**8.9**	**2.4**	**2.6**	**2.1**	**18.7**	**18.2**	**19.3**
Starch roots, potatoes	1.7	1.5	1.9	0.3	0.2	0.3	3.9	3.6	4.4
Pasta	4.6	4.5	4.8	0.8	0.8	0.8	10.7	10.4	11.0
Rice and other cereals	1.8	2.0	1.5	0.8	1.0	0.4	3.3	3.5	3.1
Flour	0.6	0.6	0.7	0.6	0.6	0.6	0.8	0.7	0.8
Vegetables	**3.3**	**3.0**	**3.6**	**0.9**	**1.0**	**0.9**	**6.9**	**6.3**	**7.8**

Vegetables excluding potatoes	3.0	2.9	3.3	0.9	1.0	0.9	6.4	5.9	7.0
Meat substitutes and vegetarian products	0.2	0.1	0.3	0.0	0.0	0.0	0.5	0.4	0.8
Legume, soy products, soy drinks	**1.4**	**1.1**	**1.7**	**0.0**	**0.1**	**0.1**	**3.5**	**2.7**	**4.2**
Pulses (excluding fresh peas, sweet corn and broad bean)	0.9	0.9	0.9	0.0	0.1	0.0	2.3	2.3	2.2
Soya beverages	0.5	0.2	0.8	0.0	0.0	0.0	1.2	0.5	2.0
Desserts and puddings soya based	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fruits	**1.1**	**1.0**	**1.3**	**0.0**	**0.0**	**0.0**	**2.7**	**2.4**	**3.1**
Fruits	1.0	0.9	1.2	0.0	0.0	0.0	2.7	2.3	3.0
Olives, avocado	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1
Milk, milk products, cheese	**17.2**	**17.0**	**17.5**	**26.5**	**26.0**	**27.2**	**2.7**	**2.7**	**2.7**
White milk and buttermilk	6.7	6.8	6.3	10.6	10.8	10.1	0.6	0.6	0.6
Yogurt and fromage blanc (quark)	1.6	1.4	1.8	2.6	2.3	2.9	0.1	0.1	0.1
Milk and yogurt beverages	1.8	1.7	1.8	2.4	2.3	2.5	0.9	0.9	0.8
Cheese (excluding fromage blanc (quark))	6.5	6.5	6.8	10.2	10.0	10.8	0.9	1.0	0.8
Desserts and puddings milk based (including ice cream)	0.6	0.5	0.7	0.8	0.7	0.9	0.2	0.2	0.3
Other milk products	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fat, oil, cream cheese, sour cream	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.2**	**0.3**	**0.3**	**0.3**
Butter and animal fats	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Margarine and lipids of mixed origins	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
Meat, poultry, fish, eggs, nuts	**40.1**	**40.3**	**39.5**	**61.6**	**61.5**	**61.2**	**6.6**	**6.9**	**6.3**

Meat	33.4	34.5	31.7	51.8	52.9	49.9	4.7	5.3	3.9
Fish products	4.6	3.8	5.4	7.4	6.1	8.7	0.3	0.2	0.4
Eggs	1.6	1.6	1.7	2.5	2.5	2.6	0.3	0.3	0.3
Nuts, seeds and olives	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.2	0.1
Nuts and seeds (including nut- and seed spreads)	0.5	0.3	0.6	0.0	0.0	0.0	1.2	0.8	1.6
Rest group (snacks and desserts)[a]	**8.5**	**8.3**	**8.9**	**4.1**	**4.0**	**4.4**	**15.3**	**15.0**	**15.8**
Cakes, pies, biscuits	4.2	3.8	4.6	1.5	1.3	1.7	8.3	7.7	9.0
Savoury snacks	0.6	0.6	0.6	0.1	0.0	0.1	1.3	1.4	1.3
Chocolate	2.0	2.1	2.0	1.3	1.4	1.3	3.0	3.1	3.0
Creams (including non-dairy and coffee creams)	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0
Confectionery non chocolate	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2
Other sugar products	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sauces (excluding dessert sauces)	1.4	1.5	1.5	0.9	0.9	1.0	2.3	2.4	2.2
Products for special nutritional use	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0
Sugar, honey, jam and syrup	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.2
Miecellaneous	**1.7**	**1.8**	**1.3**	**0.8**	**1.0**	**0.6**	**3.0**	**3.1**	**2.5**

[a] Rest group (snacks and desserts) was defined as energy-dense, low-nutritious foods

Appendix 9. Geographical map of China and study provinces in the China Health and Nutrition Survey (2004)

Appendix 10. Participants (n (%)) of the China Health and Nutrition Survey (2004) stratified in gender-age and dietary region-age groups* (n= 9720)

Items \ Age categories	Preschoolers (3-6 years)	Young school-aged children (7-10 years)	Adolescents (11-17 years)	Adults (18-59 years)	Old people (≥60 years)
Sex (n= 9720)			**n (%)**		
Male	193 (58.8)	160 (51.3)	538 (55.4)	2964 (47.7)	881 (47.7)
Female	135 (41.2)	152(49.8)	484 (44.6)	3247 (52.3)	966 (52.3)
Demography (n=9720)					
Urban	84 (25.6)	70 (24.4)	302 (33.1)	2060 (33.2)	725 (39.3)
Rural	244 (74.4)	242 (75.6)	720 (66.9)	4151 (66.8)	1122 (60.7)
Dietary habit region (n=9720)					
Heavy means	47 (14.3)	58 (18.6)	227 (22.1)	1318 (21.2)	300 (16.2)
Sweet but less heavy meals	34 (10.4)	42 (13.5)	90 (8.8)	717 (11.5)	272 (14.7)
Hot and spicy meals	102 (31.1)	114 (36.5)	363 (35.5)	1968 (31.7)	530 (28.7)
Flour (pasta)-rich meals	85 (25.9)	42 (13.5)	211 (20.6)	1390 (22.4)	464 (25.1)
Variety meals	60 (18.3)	56 (17.9)	131 (12.8)	818 (13.2)	281 (15.2)
			Anthropometry BMI z-score /BMI (Mean (kg/m^2) (%)) (n=9043) †		
Overweight					
Male	1.5 (11.0)	19.0 (8.8)	23.4 (7.6)	25.6 (26.6)	25.7 (26.6)
Female	1.4 (11.7)	18.8 (7.3)	23.4 (5.9)	25.7 (26.3)	25.7 (25.3)
Obesity					
Male	2.7 (18.4)	22.0 (7.5)	25.5 (2.0)	29.8 (6.3)	30.0 (7.5)
Female	3.0 (9.0)	21.4 (11.7)	26.9 (1.6)	30.2 (7.7)[b]	30.6 (12.4)
			Waist Circumference (cm (%) (n=9052) ‡		
Borderline					
Male	-	63.2 (12.5)	74.8 (16.5)	89.0 (28.2)	89.5 (28.6)
Female	-	60.5 (15.0)[a]	70.7 (13.6)[a]	83.8 (30.3)[b]	84.3 (34.4)[b]
Too large					
Male	-	72.2 (5.9)	87.1 (10.7)	99.4 (11.3)	100.7(15.6)
Female	-	66.4 (8.6)[b]	79.7(14.5)[a]	95.2 (13.1)[b]	96.5(26.7)[b]

*China was divided into 5 regions with different types of diet: heavy meals (Liaoning and Heilongjiang), sweet but less heavy meals (Jiangsu), hot and spicy meals (Hunan, Hubei and Guizhou), flour (pasta)-rich meals (Shandong and Henan), and variety meals (Guangxi).

† The cut-off points of BMI categories are defined according to International Obesity Task Force cut-offs (IOTF) for preschoolers; for participates at school-age (7-17) according to Group of China obesity task force,2014; for adults aged ≥ 18 according to Chinese Obesity guideline.

‡The cut-off points of waist circumference categories are defined for participants aged school-age (7-17) according to Ma GS,2010; for adults aged ≥ 18 according to Chinese Obesity guideline.

[a] *P* value of intakes for mean differences between males and females, $P \leq 0.001$ (Student's *t* test and Mann–Whitney *U* test).

[b] *P* value of intakes for mean differences between males and females, $P < 0.05$ (Student's *t* test and Mann–Whitney *U* test).

Appendix 11. Mean (SD) daily intakes of energy, proteins (total, plant, and animal), and fibers (total and insoluble) and the energy contribution of total and plant proteins among the China Health and Nutrition Survey (2004) participants stratified in sex–age groups (n= 9720)

Items \ Age categories	Preschoolers (3-6 years)	Young school-age children (7-10 years)	Adolescents (11-17 years)	Adults (18-59 years)	Old people (≥60 years)
Male			**Mean (SD)**		
Energy (kcal/d)	1274.3 (604.9)	1699.1 (610.7)	2219.7 (730.7)	2524.6 (725.6)	2245.4 (754.0)
Total protein (g/d)	38.3 (18.7)	52.5 (20.4)	68.1 (26.5)	76.0 (25.8)	68.4 (26.8)
Animal protein (g/d)	12.3 (12.6)	15.7 (15.2)	20.1 (18.6)	21.0 (18.5)	19.2 (17.0)
Plant protein (g/d)	26.0 (14.0)	36.8 (13.9)	48.0 (19.7)	55.0 (21.0)	49.2 (21.8)
Energy intake (%) of total protein	12.3 (2.9)	12.5 (2.8)	12.4 (2.8)	12.2 (2.7)	12.3 (2.9)
Energy intake (%) of plant protein	8.4 (2.5)	9.0 (2.5)	8.8 (2.5)	8.8 (2.4)	8.9 (2.6)
Total fiber (g/d)	9.2 (6.8)	12.4 (6.3)	17.2 (14.1)	19.3 (16.3)	18.3 (12.5)
Insoluble fiber (g/d)	5.9 (4.4)	7.9 (4.1)	11.0 (8.8)	12.4 (10.2)	11.7 (7.9)
Fiber g /(1000kcal*d)	7.5 (4.1)	7.6 (3.4)	7.9 (6.5)	7.7 (5.2)	8.1 (4.1)
Female					
Energy (kcal/d)	1289.5 (621.8)	1546.5 (528.0)[b]	1893.5 (607.6)[a]	2153.3 (660.0)[a]	1917.6 (716.7)[a]
Total protein (g/d)	40.3 (25.1)	48.0 (18.8)[b]	58.2 (22.1)[a]	65.5 (23.0)[a]	58.7 (23.7)[a]
Animal protein (g/d)	14.7 (21.8)	15.7 (14.8)	17.0 (16.4)[b]	18.1 (16.5)[a]	16.0 (14.2)[a]
Plant protein (g/d)	25.5 (11.2)	32.3 (13.0)[b]	41.2 (16.2)[a]	47.4 (18.2)[a]	42.7 (19.9)[a]
Energy intake (%) of total protein	12.5 (2.4)	12.5 (3.2)	12.4 (2.8)	12.3 (2.8)[b]	12.4 (2.9)
Energy intake (%) of plant protein	8.3 (2.6)	8.6 (2.6)	8.9 (2.6)	8.9 (2.4)	9.0 (2.6)
Total fiber (g/d)	8.7 (5.1)	11.3 (5.3)	14.9 (10.2)[b]	17.5 (12.7)[a]	16.1 (11.3)[a]
Insoluble fiber (g/d)	5.6 (3.5)	7.1 (3.3)[b]	9.5 (6.1)[b]	11.1 (7.9)[a]	10.3 (7.0)[a]
Fiber g /(1000kcal*d)	7.2 (3.6)	7.7 (4.1)	8.2 (5.1)	8.3 (5.0)[a]	8.4 (4.3)

SD, standard deviation

[a] *P* value of intakes for mean differences between males and females, $P \leq 0.001$ (Student's *t* test and Mann–Whitney *U* test)

[b] *P* value of intakes for mean differences between males and females, $P < 0.05$ (Student's *t* test and Mann–Whitney *U* test)

Appendix 12. Mean (SD) daily intakes of energy, proteins (total, plant, and animal), and fibers (total and insoluble) and the energy contribution of total and plant proteins among the China Health and Nutrition Survey (2004) participants stratified in dietary region-age groups* (n= 9720)

Items \ Age categories	Preschoolers (3-6 years)	Young school-age children (7-10 years)	Adolescents (11-17 years)	Adults (18-59 years)	Old people (≥60 years)
Heavy meals (incl. energy-dense foods and oil)					
Energy (kcal/d)	1195.6 (541.1)	1574.0 (673.3)	1926.2 (652.7)	2125.1 (631.9)	1992.5 (725.8)
Total protein (g/d)	34.5 (17.4)	45.1 (20.1)	56.2 (21.1)	62.2 (23.1)	60.8 (26.5)
Animal protein (g/d)	12.5 (12.8)	12.3 (13.3)	15.3 (15.4)	15.5 (15.5)	19.1 (16.8)
Plant protein (g/d)	22.0 (8.8)	32.9 (12.6)	40.9 (14.3)	46.7 (17.3)	41.7 (17.1)
Energy intake (%) of total protein	11.5 (2.1)	11.7 (3.1)	11.7 (2.5)	11.8 (2.7)	12.2 (3.0)
Energy intake (%) of plant protein	7.7 (2.0)	8.8 (2.6)	8.7 (2.0)	8.9 (2.1)	8.5 (2.2)
Total fiber (g/d)	8.2 (6.1)	11.7 (6.1)	14.7 (7.5)	17.6 (9.9)	17.6 (10.4)
Insoluble fiber (g/d)	5.3 (3.9)	7.5 (3.9)	9.4 (4.5)	11.1 (6.1)	11.1 (6.4)
Fiber g /(1000kcal*d)	6.9 (2.9)	8.2 (5.1)	8.2 (5.5)	8.5 (4.2)	8.8 (3.9)
Sweet but less heavy meals					
Energy (kcal/d)	1585.2 (811.7)	1839.2 (704.9)	2485.2 (763.8)	2645.9 (786.5)	2483.1 (829.3)
Total protein (g/d)	52.5 (25.2)	62.6 (24.0)	79.9 (28.5)	81.6 (25.0)	76.4 (29.4)
Animal protein (g/d)	23.4 (18.3)	28.6 (18.4)	33.4 (24.3)	28.4 (17.3)	23.6 (16.7)
Plant protein (g/d)	29.1 (20.3)	34.0 (15.0)	46.5 (16.5)	53.2 (21.1)	52.8 (26.0)
Energy intake (%) of total protein	13.7 (3.6)	14.0 (3.3)	13.2 (3.6)	12.6 (2.8)	12.5 (3.0)
Energy intake (%) of plant protein	7.2 (2.4)	7.5 (2.0)	7.6 (2.0)	8.1 (2.0)	8.5 (2.7)
Total fiber (g/d)	10.1 (7.9)	12.8 (7.8)	17.1 (7.6)	19.4 (10.4)	21.9 (15.7)
Insoluble fiber (g/d)	6.6 (5.5)	8.2 (5.3)	11.1 (5.2)	12.3 (6.7)	13.8 (9.9)

Fiber g /(1000kcal*d)	6.4 (3.3)	7.0 (2.9)	7.2 (3.6)	7.4 (3.4)	8.9 (4.8)
Hot and spicy meals					
Energy (kcal/d)	1217.7 (660.2)	1620.3 (562.3)	2094.9 (693.2)	2388.1 (700.8)	2061.7 (711.6)
Total protein (g/d)	39.2 (28.1)	50.6 (18.9)	65.8 (24.6)	74.6 (25.1)	64.8 (25.0)
Animal protein (g/d)	13.5 (24.2)	13.6 (13.9)	18.5 (17.0)	21.5 (18.7)	16.5 (17.2)
Plant protein (g/d)	25.7 (11.3)	37.0 (15.2)	47.2 (19.1)	53.1 (20.7)	48.4 (20.1)
Energy intake (%) of total protein	12.9 (2.8)	12.7 (3.0)	12.7 (2.9)	12.7 (3.1)	12.8 (3.3)
Energy intake (%) of plant protein	9.0 (2.8)	9.4 (2.9)	9.3 (2.8)	9.1 (2.7)	9.6 (2.9)
Total fiber (g/d)	8.8 (4.6)	12.3 (5.8)	17.5 (15.5)	19.3 (20.6)	16.6 (10.4)
Insoluble fiber (g/d)	5.4 (2.7)	7.7 (3.6)	10.9 (9.6)	12.1 (12.8)	10.4 (6.3)
Fiber g /(1000kcal*d)	8.1 (4.2)	8.1 (4.0)	8.7 (7.7)	8.2 (7.0)	8.2 (4.1)
Flour (pasta)-rich meals					
Energy (kcal/d)	1260.6 (522.5)	1525.3 (545.3)	2014.8 (685.9)	2310.8 (740.0)	1956.0 (737.5)
Total protein (g/d)	37.9 (14.8)	47.9 (19.0)	60.6 (25.6)	69.7 (25.6)	59.6 (24.3)
Animal protein (g/d)	9.2 (8.5)	10.6 (13.1)	11.4 (11.9)	13.5 (16.2)	12.1 (10.9)
Plant protein (g/d)	28.7 (13.6)	37.3 (14.0)	49.2 (21.7)	56.3 (21.2)	47.5 (22.8)
Energy intake (%) of total protein	12.3 (2.2)	12.7 (2.6)	12.1 (2.4)	12.2 (2.2)	12.4 (2.4)
Energy intake (%) of plant protein	9.2 (2.3)	10.0 (2.1)	9.9 (2.6)	9.9 (2.3)	9.7 (2.4)
Total fiber (g/d)	10.7 (7.5)	13.2 (5.0)	17.8 (8.2)	20.5 (11.7)	18.5 (12.8)
Insoluble fiber (g/d)	7.1 (5.0)	8.7 (3.3)	11.8 (8.2)	13.6 (7.4)	12.2 (8.1)
Fiber g /(1000kcal*d)	8.6 (4.4)	8.9 (2.4)	8.8 (4.1)	9.0 (4.0)	9.4 (4.2)
Variety meals					
Energy (kcal/d)	1309.5 (519.6)[b]	1600.0 (345.1)	2016.7 (618.7)[a]	2279.9 (660.0)[b]	1983.0 (669.8)[b]

Total protein (g/d)	36.8 (14.1)[b]	47.6 (13.9)[a]	62.4 (22.9)[a]	65.6 (20.2)[b]	56.9 (19.1)[b]
Animal protein (g/d)	13.7 (9.4)[a]	17.6 (11.8)[a]	26.2 (16.9)[a]	23.4 (14.5)[b]	20.9 (14.1)[b]
Plant protein (g/d)	23.1 (10.6)[b]	30.1 (7.3)	36.2 (14.0)[a]	42.2 (13.4)[b]	36.0 (12.7)[b]
Energy intake (%) of total protein	11.6 (2.6)[a]	11.9 (2.3)[a]	12.5 (3.0)[a]	11.7 (2.4)[b]	11.7 (2.5)[b]
Energy intake (%) of plant protein	7.2 (1.7)[a]	7.5 (1.1)[a]	7.2 (1.3)[a]	7.5 (1.2)[a]	7.7 (1.7)[a]
Total fiber (g/d)	7.0 (4.7)[c]	9.4 (3.4)[d]	11.3 (8.3)[a]	12.7 (7.2)[a]	11.0 (7.0)[a]
Insoluble fiber (g/d)	4.5 (2.9)[c]	5.9 (2.0)[a]	7.1 (4.8)[a]	8.0 (4.2)[d]	6.9 (4.1)[d]
Fiber g /(1000kcal*d)	5.4 (2.4)[c]	5.9 (2.0)[a]	5.5 (3.2)[a]	5.6 (2.4)[c]	5.5 (2.5)[c]

SD, standard deviation

*China was divided into 5 regions with different types of diet: heavy meals (Liaoning and Heilongjiang), sweet but less heavy meals (Jiangsu), hot and spicy meals (Hunan, Hubei and Guizhou), flour (pasta)-rich meals (Shandong and Henan), and variety meals (Guangxi).

[a] *P* for trend based on dietary culture categories, $P \leq 0.001$ (Kruskal-wallis test)

[b] *P* for trend based on dietary culture categories, $P<0.05$ (Kruskal-wallis test)

Appendix 13. Mean (SD) daily intakes of energy, proteins (total, plant, and animal), and fibers (total and insoluble) and the energy contribution of total and plant proteins among the China Health and Nutrition Survey (2004) participants stratified in urban/rural and age groups

Items \ Age categories	Preschoolers (3-6 years)	Young school-age children (7-13 years)	Adolescents (14-17 years)	Adults (18-59 years)	Old people (≥60 years)
Urban			**Mean (SD)**		
Energy (kcal/d)	1430.8 (611.2)	1943.7 (743.3)	2239.3 (732.2)	2315.6 (754.7)	2043.5 (774.8)
Total protein (g/d)	44.6 (18.5)	63.5 (27.4)	73.0 (26.0)	73.6 (26.7)	66.3 (28.5)
Animal protein (g/d)	19.0 (12.5)	25.9 (18.7)	28.9 (19.8)	26.7 (19.3)	23.4 (16.5)
Plant protein (g/d)	25.6 (13.7)	37.6 (19.0)	44.1 (17.0)	46.9 (18.7)	42.8 (20.6)
Energy intake (%) of total protein	12.8 (2.7)	13.3 (3.5)	13.3 (3.1)	13.0 (3.1)	13.1 (3.2)
Energy intake (%) of plant protein	7.2 (2.2)	7.8 (2.4)	8.0 (2.2)	8.3 (2.3)	8.5 (2.6)
Total fiber (g/d)	8.7 (6.7)	14.7 (20.5)	15.7 (8.9)	17.6 (20.2)	16.8 (12.9)
Insoluble fiber (g/d)	5.7 (4.5)	9.3 (12.6)	10.0 (5.5)	11.1 (12.5)	10.7 (8.0)
Fiber g /(1000kcal*d)	6.2 (3.2)	7.6 (9.8)	7.2 (3.4)	7.7 (6.8)	8.1 (4.3)
Rural					
Energy (kcal/d)	1228.8 (603.6)[b]	1784.2 (598.7)[b]	2136.6 (711.2)	2337.8 (696.6)	2093.7 (737.6)
Total protein (g/d)	37.3 (22.3)[a]	53.9 (20.2)[a]	63.3 (25.3)[a]	69.0 (23.9)[a]	61.5 (23.5)[a]
Animal protein (g/d)	11.4 (17.9)[a]	13.8 (13.7)[a]	15.2 (16.2)[a]	15.8 (15.3)[a]	13.7 (13.8)[a]
Plant protein (g/d)	25.9 (12.7)	40.1 (15.8)	48.1 (19.6)[b]	53.1 (20.2)[a]	47.8 (21.2)[a]
Energy intake (%) of total protein	12.2 (2.7)	12.2 (2.7)[a]	11.9 (2.4)[a]	11.9 (2.5)[a]	11.9 (2.6)[a]
Energy intake (%) of plant protein	8.8 (2.5)[a]	9.2 (2.6)[a]	9.2 (2.5)[a]	9.2 (2.4)[a]	9.3 (2.6)[a]
Total fiber (g/d)	9.1 (6.0)	14.2 (8.3)	16.6 (9.6)	18.7 (10.6)[b]	17.4 (11.3)
Insoluble fiber (g/d)	5.9 (3.9)	9.0 (5.2)	10.7 (6.1)	12.0 (6.7)[a]	11.1 (7.1)
Fiber g /(1000kcal*d)	7.8 (4.0)[a]	8.2 (4.0)	8.2 (5.2)b	8.2 (4.0)[a]	8.4 (4.1)

SD, standard deviation

[a] P value of intakes for mean differences between urban and rural, $P \leq 0.001$ (Student's *t* test and Mann–Whitney *U* test).

[b] P value of intakes for mean differences between urban and rural, $P < 0.05$ (Student's *t* test and Mann–Whitney *U* test).

Appendix 14. Mean contribution (SD) of main food groups to total protein intake (%) among the China Health and Nutrition Survey (2004) participants stratified in the sex–age groups (n= 9720)

Food groups	Age		
	3-6	**7-17**	**≥18**
Male		**% (SD)**	
Meat and meat products	16.3 (15.5)	17.5 (15.8)	16.4 (14.4)
Dairy products	2.7 (6.8)	1.0 (3.2)	0.643 (2.4)
Egg	6.8 (9.8)	4.2 (5.4)	4.4 (6.0)
Fish	3.5 (7.3)	4.2 (7.6)	4.6 (7.7)
Cereals and cereal products	48.6 (20.2)	51.1 (18.0)	51.6 (18.7)
Potatoes	1.2 (2.2)	1.1 (2.2)	1.0 (2.2)
Legumes	8.3 (11.2)	8.1 (10.7)	8.6 (11.5)
Vegetables	7.0 (5.3)	7.4 (4.8)	7.9 (5.0)
Fruits	0.289 (0.753)	0.164 (0.490)	0.121 (0.423)
Others	5.4 (8.4)	5.3 (7.7)	4.7 (6.7)
Female			
Meat and meat products	17.8 (15.7)	16.2 (14.9)	15.6 (14.3)
Dairy products	2.9 (7.6)	1.5 (4.5)	0.823 (3.0)
Egg	7.3 (10.7)	4.8 (6.1)	4.9 (6.5)
Fish	4.1 (8.2)	4.3 (7.2)	4.4 (7.6)
Cereals and cereal products	47.5 (18.7)	50.1 (19.2)	51.2 (18.7)
Potatoes	0.785 (1.5)	1.1 (2.2)	1.2 (2.9)
Legumes	6.8 (10.3)	8.4 (11.2)	8.7 (11.8)
Vegetables	6.8 (5.4)	7.8 (5.6)	8.3 (8.3)
Fruits	0.305 (0.623)	0.205 (0.579)	0.181 (0.573)
Others	5.7 (8.6)	5.6 (7.6)	4.6 (6.6)

SD, standard deviation

Appendix 15. Mean contribution (SD) of main food groups to total dietary fiber intake (%) among the China Health and Nutrition Survey (2004) participants, stratified by the sex–age groups (n= 9720)

Food groups	**Age**		
	3-6	**7-17**	**≥18**
Male		**% (SD)**	
Cereals and cereal products	38.0 (19.8)	38.8 (18.3)	38.5 (19.1)
Potatoes	3.2 (5.6)	2.7 (4.7)	2.5 (4.8)
Legumes	7.9 (15.6)	6.8 (12.6)	7.1 (13.6)
Vegetables	38.6 (20.7)	41.3 (19.6)	43.1 (19.5)
Fruits	4.6 (11.4)	2.5 (7.0)	1.8 (5.8)
Others	7.6 (13.5)	7.8 (13.5)	6.9 (12.9)
Female			
Cereals and cereal products	39.3 (18.9)	36.4 (18.8)	37.4 (19.4)
Potatoes	2.9 (5.7)	2.6 (4.4)	2.7 (5.4)
Legumes	6.8 (14.2)	7.1 (14.1)	7.1 (13.9)
Vegetables	36.6 (19.4)	42.2 (19.8)	43.4 (19.7)
Fruits	5.2 (10.7)	3.0 (8.4)	2.6 (7.0)
Others	9.2 (14.5)	8.6 (15.2)	6.8 (12.8)

SD, standard deviation

Appendix 16. Generalized linear model for the associations between BMI and animal and plant protein intakes among participants of the Belgian national food consumption survey (2004-2005)

BMI (kg/m2)	Coefficients		95% Confidence Interval		
	B	**SE**	**Lower Bound**	**Upper Bound**	***P***
Males (n= 1535)†‡					
Animal protein	0.014	0.004	0.006	0.022	<0.001
Plant protein	0.010	0.022	-0.034	0.053	0.665
Age (years)					
15-18	-2.3	0.788	-3.8	-0.746	0.004
19-59	0.960	0.766	-0.540	2.5	0.210
60-74	2.7	0.776	1.2	4.3	<0.001
Education					
Lower secondary or less	0.522	0.250	0.032	1.011	0.037
Vocational, technical or art	0.054	0.252	-0.440	0.549	0.830
General secondary	-0.064	0.296	-0.643	0.515	0.829
Region					
Flanders	-0.530	0.197	-0.916	-0.144	0.007
Brussels	-1.0	0.371	-1.7	-0.276	0.007
Females (n= 1519)§					
Animal protein	0.001	0.006	-0.012	0.013	0.886
Plant protein	-0.092	0.028	-0.147	-0.037	0.001
Age (years)	-3.2	0.359	-3.9	-2.5	<0.001
15-18	-0.596	0.320	-1.2	0.031	0.062
19-59	1.3	0.293	0.755	1.9	<0.001
60-74					
Education					
Lower secondary or less	2.0	0.297	1.4	2.6	<0.001
Vocational, technical or art	1.4	0.313	0.766	2.0	<0.001
General secondary	0.467	0.339	-0.196	1.1	0.168
Region					
Flanders	-1.7	0.690	-3.1	-0.377	0.012
Brussels	-1.4	1.0	-3.4	0.607	0.172

SE, standard error of B coefficient

† Age (≥75 years), higher education and Walloon are as reference category

‡Significantly associated interaction: Age group (15-18 years) * total plant protein: B=-0.064, *P*=0.019; Age group (60-74 years) * total plant protein : B=-0.057, *P*=0.047

§Significantly associated interaction: Region (Flanders) * total plant protein: B=0.020, *P*=0.019

Appendix 17. Generalized linear model for the associations between waist circumference and animal and plant protein intakes among participants of the Belgian national food consumption survey (2004-2005)

Waist circumference (cm)*†	**Coefficients**		**95% Confidence Interval**		
	B	**SE**	**Lower Bound**	**Upper Bound**	***P***
Males (n= 1535)‡					
Animal protein	0.074	0.026	0.023	0.125	0.004
Plant protein	-0.130	0.031	-0.190	-0.071	<0.001
Age (years)	-19.0	0.966	-20.9	-17.1	<0.001
15-18	-7.6	0.903	-9.3	-5.8	0.000
19-59	0.428	0.846	-1.2	2.1	0.613
60-74					
Education					
Lower secondary or less	1.8	2.2	-2.5	6.2	0.411
Vocational, technical or art	3.6	2.1	-0.447	7.7	0.081
General secondary	0.857	2.5	-4.0	5.7	0.728
Region					
Flanders	-1.7	0.654	-3.0	-0.462	0.008
Brussels	-2.8	1.2	-5.2	-0.410	0.022
Females (n= 1519)§					
Animal protein	0.002	0.007	-0.011	0.015	0.723
Plant protein	-0.081	0.029	-0.139	-0.023	0.006
Age (years)	-3.4	0.376	-4.1	-2.7	<0.001
15-18	-0.708	0.340	-1.4	-0.042	0.037
19-59	1.3	0.309	0.656	1.9	<0.001
60-74					
Education					
Lower secondary or less	1.7	0.312	1.1	2.3	<0.001
Vocational, technical or art	1.4	0.328	0.723	2.0	<0.001
General secondary	0.353	0.351	-0.335	1.0	0.315
Region					
Flanders	-2.0	0.721	-3.4	-0.559	0.006
Brussels	-1.6	1.1	-3.6	0.480	0.133

SE, standard error of B coefficient

† Age (≥75 years), higher education and Walloon are as reference category

‡Significantly associated interaction: Education (Vocational, technical or art) * total animal protein: B=-0.069, *P*=0.036

§Significantly associated interaction: Region (Flanders) * total plant protein: B=0.081, P=0.016

Appendix 18. Association of animal and plant protein, and specific food group protein with education, geography by Generalized Linear Model among the Belgian population (n = 3083)

	Animal protein and specific main sources																			
Predictor variables† µ	**Total animal protein**				**Dairy protein§**				**Meat protein‡**				**Fish protein**				**Egg protein**			
	B	**95 % CI**		***P***	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***
Education																				
Lower secondary or less	5.0	-0.482	10.6	0.074	-0.102	-0.139	-0.065	<0.001	0.037	0.007	0.067	0.017	0.363	0.141	0.585	0.001	0.127	0.042	0.212	0.003
Vocational, technical or art education	-5.4	-9.0	-1.8	0.003	-0.110	-0.149	-0.071	<0.001	0.032	0.002	0.063	0.040	0.116	-0.021	0.254	0.098	0.043	-0.044	0.131	0.329
General secondary education	-3.4	-7.1	0.3	0.075	-0.055	-0.098	-0.013	0.010	0.002	-0.032	0.035	0.912	0.052	-0.086	0.189	0.463	0.015	-0.080	0.111	0.751
Geography																				
Flanders	-5.2	-8.1	-2.2	0.001	-0.009	-0.040	0.022	0.566	-0.036	-0.060	-0.013	0.003	0.106	-0.012	0.223	0.078	-0.033	-0.105	0.039	0.365
Brussels	-1.8	-7.1	3.5	0.502	-0.034	-0.090	0.021	0.226	-0.057	-0.100	-0.013	0.010	0.077	-0.119	0.274	0.440	-0.045	-0.173	0.083	0.489
	Plant protein and specific main sources																			
Education	**Total plant protein**				**Cereal protein**				**Potato and other tubers**				**Vegetable Protein**				**Fruit Protein**			
Lower secondary or less	-2.3	-4.7	-0.043	0.046	-0.019	-0.041	0.003	0.092	0.096	0.066	0.125	<0.001	0.098	-0.040	0.237	0.164	0.243	0.035	0.451	0.022
Vocational, technical or art education	-2.4	-4.0	-0.840	0.003	-0.022	-0.044	0.001	0.057	0.079	0.048	0.109	<0.001	-0.193	-0.282	-0.104	<0.001	-0.016	-0.146	0.114	0.810
General secondary education	-0.334	-1.9	1.3	0.686	-0.004	-0.028	0.020	0.747	0.040	0.007	0.073	0.018	-0.120	-0.212	-0.028	0.010	0.028	-0.102	0.157	0.677
Geography																				
Flanders	2.5	1.3	3.7	<0.001	0.022	-0.017	0.060	0.265	0.079	0.021	0.138	0.008	0.083	0.037	0.129	<0.001	0.184	0.074	0.293	0.001
Brussels	2.8	0.678	5.0	0.010	0.057	-0.012	0.127	0.108	0.045	-0.065	0.155	0.421	0.131	0.051	0.212	0.001	0.334	0.147	0.522	<0.001

†GLM was used to investigate the associations of protein intakes (dependent variables) with education level and geography controlling for gender, age, energy intake and interactions (gender*age, education*age, education*gender, geography*age, geography*gender).

‡ Meat protein includes meat, poultry and processed meat.

µ High education and Walloon were as reference

Appendix 19. Association of total fiber and specific food group fiber with education and geography by Generalized Linear Model among the Belgian population

Predictor variables†‡	Total fiber				Cereal fiber				Potato and other tubers fiber				Vegetable fiber				Fruit fiber			
	B	95 % CI		*P*	B	95 % CI		*P*	B	95 % CI		*P*	B	95 % CI		*P*	B	95 % CI		*P*
Education																				
Lower secondary or less	-2.0	-2.9	-1.1	<0.001	-0.051	-0.085	-0.017	0.003	0.098	0.067	0.128	<0.001	0.072	-0.068	0.211	0.314	0.130	-0.043	0.304	0.140
Vocational, technical or art education	-1.5	-2.4	-0.502	0.003	-0.069	-0.106	-0.031	0.000	0.074	0.043	0.105	<0.001	-0.198	-0.287	-0.108	<0.001	-0.054	-0.162	0.053	0.322
General secondary education	-0.190	-1.2	0.810	0.710	-0.042	-0.081	-0.003	0.036	0.036	0.002	0.071	0.037	-0.109	-0.201	-0.017	0.021	0.055	-0.052	0.163	0.311
Geography																				
Flanders	3.7	2.5	4.8	<0.001	0.150	0.130	0.170	0.000	0.085	0.025	0.145	0.005	0.183	0.104	0.262	<0.001	0.106	0.055	0.158	<0.001
Brussels	2.7	0.616	4.8	0.011	0.001	-0.036	0.038	0.963	0.049	-0.064	0.162	0.396	0.200	0.059	0.342	0.006	0.104	0.016	0.192	0.020

†GLM was used to investigate the associations of protein intakes (dependent variables) with education level and geography controlling for gender, age, energy intake and interactions (gender*age, education*age, education*gender, geography*age, geography*gender).

‡ High education and Walloon were as reference.

Appendix 20. Association of total, animal and plant protein with socio-economic status factors among European adolescents

Predictor variables[†‡]	Total protein				Animal protein				Plant protein			
	B	**95 % CI**		***P***	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***
Education of mother												
Lower and lower secondary education	0.706	-1.7	3.1	0.569	1.8	-0.719	4.3	0.162	-0.592	-1.7	0.557	0.313
Higher secondary education	-0.502	-2.6	1.6	0.646	1.5	-0.741	3.7	0.191	-1.4	-2.4	-0.398	0.006
Education of father												
Lower and lower secondary education	-0.111	-2.4	2.2	0.924	-0.897	-3.3	1.5	0.457	0.311	-0.768	1.4	0.573
Higher secondary education	-0.614	-2.8	1.5	0.577	-1.7	-4.0	0.513	0.131	0.860	-0.166	1.9	0.100
Parental employment												
Both unemployed parents	-1.7	-5.2	1.9	0.365	-2.9	-6.6	0.816	0.126	1.4	-0.324	3.1	0.113
One employed parent	-1.1	-2.8	0.650	0.219	-2.1	-3.9	-0.330	0.020	0.550	-0.276	1.4	0.192

[†]GLM was used to investigate the associations of protein intakes (dependent variables) with socio-economic status (parental education level and parental employment) controlling for gender, age, energy intake, physical activity, country center and interactions (gender*age, gender*physical activity, age*physical activity).

[‡] Higher education or university degree of parents and both employed parents are as reference.

Appendix 21. Association of total fiber, water-soluble fiber and water-insoluble fiber with socio-economic status factors among European adolescents

	Total fiber				Water-soluble fiber				Water-insoluble fiber			
Predictor variables[†‡]	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***
Education of mother												
Lower and lower secondary education	-0.717	-1.397	-0.037	0.039	-0.193	-0.404	0.019	0.075	-0.540	-1.0	-0.043	0.033
Higher secondary education	-0.314	-0.913	0.285	0.304	-0.178	-0.365	0.009	0.062	-0.227	-0.664	0.211	0.310
Education of father												
Lower and lower secondary education	-1.1	-1.7	-0.433	0.001	-0.362	-0.561	-0.163	<0.001	-0.713	-1.2	-0.246	0.003
Higher secondary education	-0.027	-0.631	0.578	0.931	-0.008	-0.196	0.181	0.938	0.071	-0.370	0.512	0.752
Parental employment												
Both unemployed parents	-0.272	-1.3	0.719	0.591	-0.033	-0.344	0.277	0.833	-0.252	-0.974	0.470	0.494
One employed parent	0.166	-0.324	0.655	0.507	0.130	-0.023	0.282	0.096	0.047	-0.309	0.404	0.795

[†]GLM was used to investigate the associations of protein intakes (dependent variables) with socio-economic status (parental education level and parental employment) controlling for gender, age, energy intake, physical activity, country center and interactions (gender*age, gender*physical activity, age*physical activity).
[‡] Higher education or university degree of parents and both employed parents are as reference.

Appendix 22. Association of animal protein, specific food group animal protein with socio-economic status factors by Generalized Linear Model† among the Chinese population

	Total animal protein				Meat protein§				Dairy protein§				Fish protein				Egg protein			
Predictor variables‡	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***
Preschoolers[µ]																				
Education of mother																				
Lower and lower secondary education	-11.9	-20.2	-3.6	0.005	-13.5	-18.6	-8.4	<0.001	0.677	-1.382	2.7	0.519	0.553	-2.4	3.5	0.712	-0.258	-3.2	2.7	0.865
Secondary education	-4.6	-13.3	4.1	0.299	-9.4	-14.8	-4.0	0.001	1.3	-0.847	3.5	0.230	1.0	-2.1	4.1	0.521	1.3	-1.8	4.5	0.401
Parental employment																				
Both unemployed parents	0.237	-6.1	6.6	0.942	1.2	-2.5	5.0	0.517	-2.2	-3.8	-0.544	0.009	-0.398	-2.6	1.8	0.722	2.3	0.116	4.6	0.039
One employed parent	3.2	-0.135	6.5	0.060	1.4	-0.706	3.5	0.195	-0.362	-1.2	0.470	0.394	-0.578	-1.7	0.591	0.332	2.2	0.993	3.4	<0.001
School aged children																				
Education of mother	-6.4	-14.6	1.8	0.123	-3.9	-10.6	2.7	0.246	-2.5	-3.7	-1.2	0.000	1.5	-1.4	4.4	0.303	-1.6	-3.7	0.468	0.130
Lower and lower secondary education	0.094	-8.1	8.3	0.982	0.357	-6.3	7.0	0.916	-1.9	-3.2	-0.662	0.003	2.4	-0.510	5.2	0.107	-1.0	-3.1	1.0	0.327
Secondary education																				
Education of father	-7.7	-13.2	-2.2	0.006	-2.0	-6.4	2.5	0.391	-0.604	-1.4	0.233	0.157	-3.7	-5.6	-1.7	<0.001	-2.1	-3.5	-0.694	0.003
Lower and lower secondary education	-5.5	-11.1	0.079	0.053	-1.2	-5.7	3.3	0.599	-0.186	-1.0	0.661	0.667	-3.5	-5.4	-1.5	<0.001	-1.4	-2.8	0.000	0.050
Secondary education																				
Parental employment	-3.2	-10.7	4.4	0.415	-0.509	-6.7	5.6	0.871	-0.404	-1.6	0.749	0.492	-1.0	-3.7	1.6	0.443	-0.393	-2.3	1.5	0.687
Both unemployed parents	4.2	1.4	7.1	0.004	3.8	1.5	6.1	0.001	-0.017	-0.451	0.418	0.940	0.757	-0.244	1.8	0.138	0.031	-0.688	0.751	0.933
One employed parent																				
Adults																				
Education																				

Lower middle school	-9.3	-10.9	-7.7	<0.001	-4.7	-5.9	-3.4	<0.001	-0.963	-1.2	-0.729	<0.001	-2.1	-2.7	-1.4	<0.001	-1.2	-1.6	-0.711	<0.001
Upper middle school, technical education	-3.6	-5.3	-1.9	<0.001	-0.980	-2.3	0.319	0.139	-0.568	-0.810	-0.326	<0.001	-1.3	-2.0	-0.580	<0.001	-0.264	-0.740	0.211	0.276
Employment																				
Unemployed	-2.9	-4.2	-1.6	<0.001	-0.133	-1.2	0.888	0.798	-0.507	-0.720	-0.294	<0.001	-0.781	-1.3	-0.224	0.006	-1.0	-1.4	-0.602	<0.001
Employed	-3.9	-5.0	-2.8	<0.001	-1.1	-2.0	-0.220	0.014	-0.548	-0.740	-0.357	<0.001	-0.554	-1.0	-0.076	0.023	-1.1	-1.5	-0.720	<0.001

[†]GLM was used to investigate the associations of protein intakes (dependent variables) with socio-economic status (education level and employment status) controlling for gender, age, energy intake, cluster effect and interactions (gender*age).
[‡] Higher education or university degree of parents and both employed parents are as reference for preschoolers and school-aged children. Higher education or university degree and retired status are as reference for adults

[µ] Only meat derived protein intake of preschoolers was associated with paternal education, B=-2.2, *P*=0.028.

Appendix 23. Association of plant protein and specific food group plant protein with socio-economic status factors by Generalized Linear Model† among the Chinese population

Predictor variables‡	Total plant protein				Cereal protein				Potato and other tubers				Vegetable Protein				Fruit Protein				Legume protein			
	B	95 % CI		*P*	B	95 % CI		*P*	B	95 % CI		*P*	B	95 % CI		*P*	B	95 % CI		*P*	B	95 % CI		*P*
Preschoolers [µ]																								
Education of mother																								
Lower and lower secondary education	-3.5	-9.9	2.9	0.283	0.6	-4.1	5.4	0.795	0.5	0.04	1.0	0.032	-0.6	-2.2	1.1	0.503	0.04	-0.1	0.2	0.710	-4.6	-8.9	-0.2	0.040
Secondary education	-6.4	-13.1	0.3	0.060	-1.0	-6.0	4.0	0.708	0.6	0.1	1.1	0.028	-0.7	-2.4	1.0	0.439	0.1	-0.1	0.3	0.215	-5.2	-9.9	-0.6	0.026
Parental employment																								
Both unemployed parents	5.1	0.2	10.0	0.040	-1.9	-5.4	1.7	0.311	0.2	-0.2	0.5	0.359	-0.02	-1.2	1.2	0.981	0.01	-0.1	0.1	0.880	7.3	3.8	10.7	<0.001
One employed parent	1.2	-1.3	3.8	0.345	-1.8	-3.8	0.1	0.060	0.0	-0.2	0.2	0.769	-0.2	-0.8	0.5	0.570	0.1	0.0	0.1	0.132	1.0	-0.7	2.8	0.249
School-aged children																								
Education of mother																								
Lower and lower secondary education	-0.9	-7.3	5.5	0.782	2.1	-3.1	7.3	0.432	0.1	-0.4	0.6	0.806	0.2	-1.4	1.9	0.786	0.1	-0.1	0.2	0.539	-2.2	-6.8	2.3	0.337
Secondary education	-3.8	-10.3	2.6	0.241	0.2	-5.0	5.4	0.931	-0.1	-0.6	0.4	0.612	-0.4	-2.1	1.2	0.598	0.1	-0.1	0.3	0.198	-2.1	-6.7	2.5	0.371
Education of father																								
Lower and lower secondary education	4.9	0.6	9.2	0.025	2.6	-0.9	6.1	0.145	0.3	-0.04	0.7	0.081	0.5	-0.6	1.6	0.372	-0.2	-0.3	-0.05	0.006	1.2	-1.9	4.3	0.433
Secondary education	5.4	1.1	9.8	0.015	1.2	-2.3	4.8	0.500	0.3	-0.03	0.7	0.077	0.6	-0.5	1.7	0.286	-0.1	-0.3	-0.02	0.022	2.8	-0.4	5.9	0.082
Parental employment																								
Both unemployed parents	2.7	-3.2	8.7	0.367	3.4	-1.4	8.2	0.168	-0.1	-0.6	0.3	0.544	-0.8	-2.3	0.7	0.282	0.00	-0.2	0.1	0.803	0.8	-3.4	5.0	0.697
One employed parent	-1.4	-3.7	0.8	0.206	-0.6	-2.4	1.3	0.549	0.00	-0.2	0.2	0.919	0.00	-0.6	0.6	0.999	0.00	-0.1	0.02	0.183	0.3	-1.3	1.9	0.749

													Adults											
Education																								
Lower middle school	3.1	1.6	4.6	<0.001	3.7	2.6	4.9	<0.001	0.4	0.2	0.5	<0.001	0.3	-0.1	0.7	0.116	-0.1	-0.2	-0.1	<0.001	-0.5	-1.6	0.6	0.347
Upper middle school, technical education	0.9	-0.7	2.4	0.277	0.8	-0.4	2.0	0.174	0.2	0.1	0.4	<0.001	0.04	-0.4	0.5	0.854	-0.1	-0.1	-0.04	<0.001	-0.1	-1.2	1.1	0.931
Employment																				<0.001				
Unemployed	1.2	-0.02	2.4	0.054	1.5	0.5	2.6	0.004	0.1	-0.03	0.2	0.127	-0.1	-0.4	0.3	0.680	-0.1	-0.1	-0.1	<0.001	-1.0	-1.9	-0.1	0.037
Employed	1.8	0.8	2.8	0.001	2.6	1.6	3.5	<0.001	0.1	-0.05	0.2	0.316	-0.1	-0.3	0.2	0.680	-0.1	-0.1	-0.1	<0.001	-1.5	-2.2	-0.7	<0.001

[†]GLM was used to investigate the associations of protein intakes (dependent variables) with socio-economic status (education level and employment status) controlling for gender, age, energy intake, cluster effect and interactions (gender*age).

[‡] Higher education or university degree of parents and both employed parents are as reference for preschoolers and school-aged children. Higher education or university degree and retired status are as reference for adults.

[µ] Plant protein and plant protein from food sources are not associated with paternal education.

Appendix 24. Association of fiber and specific food group fiber with socio-economic status factors by Generalized Linear Model† among the Chinese population

Predictor variables‡	Total fiber				Cereal fiber				Potato and other tubers fiber				Vegetable fiber				Fruit fiber				Legume fiber			
	B	**95 % CI**		***P***	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***	**B**	**95 % CI**		***P***
Parental employment	Preschoolers[µ]																							
Both unemployed parents	1.2	-1.5	3.8	0.396	-2.0	-3.5	-0.6	**0.005**	0.1	-0.2	0.4	0.355	0.5	-0.8	1.8	0.446	-0.4	-1.0	0.2	0.188	2.8	1.9	3.8	<0.001
One employed parent	1.1	-0.3	2.5	0.115	0.6	-0.1	1.4	0.101	0.1	-0.1	0.2	0.374	-0.9	-1.6	-0.2	0.015	-0.02	-0.3	0.3	0.868	-0.1	-0.6	0.4	0.602
Education of father	School age children‡[µ]																							
Lower and lower secondary education	1.9	-0.7	4.6	0.151	0.1	0.0	0.2	**0.047**	0.1	-0.1	0.4	0.321	0.1	-0.1	0.2	0.313	-0.4	-0.7	-0.1	0.022	-0.1	-0.4	0.2	0.639
Secondary education	2.5	-0.1	5.2	0.064	0.1	0.0	0.2	0.084	0.1	-0.2	0.3	0.548	0.1	0.0	0.2	0.223	-0.4	-0.7	0.0	0.027	0.1	-0.2	0.4	0.558
Education	Adults																							
Lower middle school	-0.2	-1.4	0.9	0.676	0.7	0.2	1.1	**0.003**	0.2	0.1	0.3	**<0.001**	0.1	-0.5	0.8	0.716	-0.5	-0.7	-0.4	<0.001	-0.3	-0.7	0.2	0.295
Upper middle school , technical education	-0.5	-1.7	0.6	0.362	0.2	-0.3	0.6	0.489	0.2	0.1	0.3	**0.002**	-0.2	-0.9	0.4	0.506	-0.3	-0.5	-0.2	<0.001	0.02	-0.5	0.5	0.928
Employment																								
Umployed	-1.0	-1.9	-0.1	**0.029**	0.2	-0.2	0.5	0.405	0.0	0.0	0.1	0.279	0.1	-0.4	0.6	0.756	-0.4	-0.5	-0.3	<0.001	-0.1	-0.5	0.4	0.824
Employed	-1.0	-1.8	-0.2	**0.012**	0.8	0.5	1.1	**<0.001**	0.0	-0.1	0.1	0.597	-0.2	-0.6	0.3	0.500	-0.3	-0.4	-0.2	<0.001	-0.3	-0.7	0.1	0.096

†GLM was used to investigate the associations of fiber intakes (dependent variables) with socio-economic status (education level and employment status) controlling for gender, age, energy intake, cluster effect and interactions (gender*age).

‡ Higher education or university degree of parents and both employed parents are as reference for preschoolers and school-aged children. Higher education or university degree and retired status are as reference for adults.

µ Dietary fiber and fiber from food sources are not associated with paternal education among preschoolers; dietary fiber and fiber from food sources are not associated with maternal education and paternal education among school aged children

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About the author

Yi LIN was born in Shanghai, China. She got her bachelor's degree in the major of applied biology from East China Normal University. After finishing bachelor's degree, she joined an international company, known as Wyeth, the Asian pacific head office based in Shanghai, China, which belongs to Nestlé and Pfizer now. She was working as commercial specialist and project coordinator for four years to deal with international and domestic affairs about nutrition and medicine issues.

Since she made her strong decision to pursue her academic dreams, she gave up promotion in the international company and stable life in Shanghai in the end of 2004 and came to Belgium for academic study in 2005. She graduated her master in food science and nutrition at Ghent university in September, 2006. In the same year, she got a chance to go for anther academic training course of public health. In July, 2007, she got her second master degree in public health methodology at Université libre de Bruxelles.

In 2010, she started her PhD at department of public health of the faculty of Medicine and Health Sciences, Ghent university. Under the supervision of Prof. De Henauw and Prof. Huybrechts, she worked on four independent research studies. One of her research studies was collaborated with Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention (CDC). She conquered the problems and succeeded to get connection to the responsible person of Institute of Nutrition and Food Safety. After she got oral permission from CDC, the proposal was developed with under the supervision of Inge Huybrechts and Stefaan De Henauw. During the collaboration, she coordinated with Institute of Nutrition and Food Safety, Chinese CDC.

During PhD, she tried to get more research experience. In 2011, she worked as researcher and project manager in Vitalstrategic Research Institute in Shanghai. She worked on outcome research, which was focusing on national clinical registry study on type 2 diabetes, cardiovascular diseases in Chinese patients. At the last stage of PhD and before internal defense, she won the highest honor from Chinese government—Chinese Government Award for Outstanding self-financed PhD Students Abroad.

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